FORMULATION, CHARACTERIZATION AND EVALUATION OF KETOCONAZOLE NIOSOMAL GEL FOR THE TREATMENT OF CUTANEOUS LEISHMANIASIS.

A Dissertation submitted to
THE TAMIL NADU Dr. M.G.R MEDICAL UNIVERSITY
CHENNAI – 600 032

in partial fulfilment of the requirements for the award of degree of

MASTER OF PHARMACY
IN
PHARMACEUTICS

submitted by
Register Number: 261411262

under the guidance of
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Associate Professor
Department of Pharmaceutics

COLLEGE OF PHARMACY
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CHENNAI – 600 003
APRIL – 2016

DEPARTMENT OF PHARMACEUTICS
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CERTIFICATE

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Gratitude is the memory of the Heart

- Jean Baptiste

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1.INTRODUCTION

NOVEL DRUG DELIVERY SYSTEM

Novel drug delivery system aims to provide a therapeutic amount of drug to the appropriate site in the body to accomplish promptly and then maintain the desired drug concentration. The drug delivery system should deliver the drug at a rate controlled by the necessity of the body over a specified term of treatment.

The two important aspects of idealized drug delivery are as follows;

- Spatial drug delivery: Targeting a drug to a particular organ or tissue.
- Temporal drug delivery: the drug delivery rate to a target tissue is controlled.

Novel drug delivery systems are designed to achieve a continuous delivery of drugs at predictable and reproducible kinetics over an extended period of time in the circulation. The potential advantages of this concept include minimization of drug related side effects due to controlled therapeutic blood levels instead of oscillating blood levels, improved patient compliance due to reduced frequency of dosing and the reduction of the total dose of drug administered. Hence, the combination of both sustained release and control release properties in a delivery system would further enhance therapeutic efficacy.[4]

A number of novel drug delivery systems has emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery. Encapsulation of the drug in vesicular structure is one such system, which can be predicted to prolong the existence of the drug in systemic circulation and reduce the toxicity if selective uptake can be achieved. Consequently a number of vesicular drug delivery systems such as liposomes, niosomes, transferosomes and pharmacosomes were developed. Advances have since been made in the area of vesicular drug delivery, leading to the development of this system that allow drug targeting and sustained or controlled release of conventional drug medicines.[10]

For many decades treatment of an acute disease or a chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical conventional dosage forms. Even today conventional drug delivery system occupies most of the part in a prescription as well as drug store. This type of drug delivery system is known to provide a prompt release of drug. But to achieve as well as to maintain the drug concentration within the

therapeutically effective range needed for treatment, it is often necessary to take this type of drug delivery system several times a day. This results in significant fluctuations in drug level.[5]

DISADVANTAGES OF CONVENTIONAL DOSAGE FORMS[52]

i. Drugs with short half- life require frequent administration, which increases chances of missing the dose of a drug leading to poor patient compliance.
ii. A typical peak- valley plasma concentration- time profile is obtained which makes attainment of steady state condition very difficult.
iii. The unavoidable fluctuations in the drug concentration may lead to under- medication or over- medication as the C_{ss} values fall or rise beyond the therapeutic range which may lead to adverse effects of the drug.
iv. Drug accumulation may occur in case of the frequent administration of the drugs.

To overcome the above drawbacks, drug delivery systems capable of controlling the rate of drug delivery, sustaining the duration of therapeutic action and/ or targeting the drug to a particular tissue was developed.

The novel drug delivery systems are diversely referred to as "controlled release", "sustained release", "zero- order", "reservoir", "monolithic", "membrane- controlled", "smart", "stealth" etc. some of these common terms are defined as follows:[1]

- **Prolonged/ sustained release**: the delivery system prolongs therapeutic blood or tissue levels of the drug for an extended period of time.
- **Zero order release**: the drug release does not vary with time; thus the delivery system maintains a (relatively) constant effective drug level in the body for prolonged periods.
- **Variable release**: the delivery system provides drug input at a variable rate, to match, for example endogenous circadian rhythms, or to mimic natural biorhythms.
- **Bio- responsive release**: the system modulates drug release in response to a biological stimulus (e.g. blood glucose levels triggering the release of insulin from a drug delivery device)
- **Modulated/ self-regulated release**: the system delivers the necessary amount of drug under the control of the patient.

- **Rate- controlled release**: the system delivers the drug at some pre- determined rate, whether systemically or locally, for a specific period of time.
- **Targeted- drug delivery**: the delivery system achieves site- specific drug delivery.
- **Temporal- drug delivery**: the control of delivery to produce an effect in a desired time-related manner.
- **Spatial- drug delivery**: the delivery of a drug to a specific region of the body (thus this term encompasses both route of administration and drug distribution).
- **Bioavailability**: the rate and extent at which a drug is taken up into the body.
- **Repeat action**: individual dose is released fairly soon after administration and second or third doses are subsequently released at intermittent intervals.[1]

CLASSIFICATION OF CONTROLLED DRUG DELIVERY SYSTEMS (CDDS).[5]

Controlled drug delivery systems can be classified as

- Rate-preprogrammed drug delivery system.
- Activation-modulated drug delivery system.
- Feed-back regulated drug delivery system.
- Site-targeting drug delivery system.

TARGETED/ SITE- SPECIFIC DRUG DELIVERY SYSTEM

While rate- controlled systems can deliver the drug at a predetermined rate, they are generally unable to control the fate of the drug, once it enters the body. The concept of designing specified delivery system to achieve selective drug targeting has been originated from the perception of Paul Ehrlich, who proposed drug delivery to be as a **"Magic bullet"**(Paul Ehrlich, 1902) describing targeted drug delivery as an event where, a drug-carrier complex/conjugate, delivers drug(s) exclusively to the preselected target cells in a specific manner.[6]

Targeted drug delivery system refers to systems that place the drug at or near the receptor site or site of action. Targeted drug delivery implies selective and effective localization of drug into the target(s) at therapeutic concentrations with limited access to target sites.[6]

Site- specific drug delivery is desirable in therapeutics, in order to improve:[1]

- *Drug safety,* as toxic side- effects caused by drug action at non- target sites are minimized.
- *Drug efficacy,* as the drug is concentrated at the site of action rather than being dispersed throughout the body.
- *Patient compliance,* as increased safety and efficacy should make therapy more acceptable and thus improve compliance.[1]

Technologies for drug targeting are concerned with delivering drugs to specific targets in the body and also to protect drugs from degradation and premature elimination. They include the use of

- *Soluble carriers,* such as monoclonal antibodies, dextrans, soluble synthetic polymers
- *Particulate carriers,* such as liposomes, niosomes, micro- and nano- particles, microspheres
- *Target- specific recognition moieties,* such as monoclonal antibodies, carbohydrates and lectins.

Recent advances in biological and chemical sciences have led to the development of various "smart" technologies to ensure more effective drug delivery and targeting of drugs to specific sites within the body. Such approaches include the use of:

- Antibody- directed enzyme/ prodrug therapy (ADEPT)[52]
- Virus- directed prodrug/ enzyme therapy (VDEPT)
- Chemical drug delivery systems

DRUG DELIVERY CARRIERS[2, 6]

A Carrier is one of the most important entities essentially required for successful transportation of the loaded drug(s). they are drug vectors, which sequester, transport and retain drug *en route*, while elute or deliver it within or in the vicinity of target.[6] Colloidal drug carrier systems such as micellar solutions, vesicle and liquid crystal dispersions, as well as nanoparticle dispersions consisting of small particles of 10–400 nm diameter show great promise as drug delivery systems. When developing these formulations, the goal is to obtain systems with optimized drug loading and release properties, long shelf-life and low toxicity.

The incorporated drug participates in the microstructure of the system, and may even influence it due to molecular interactions, especially if the drug possess amphiphilic and/or mesogenic properties.[2]

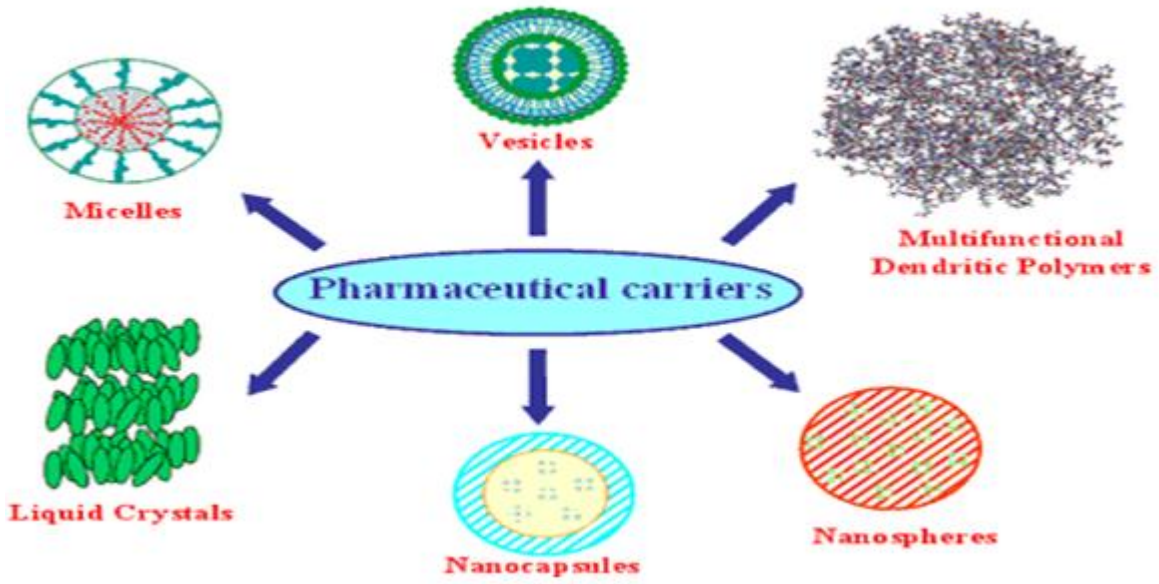


Figure 1 Pharmaceutical carriers[2]

An ideal carrier engineered as a targetable device should have the following features:

- It must be able to cross anatomical barriers and in case of tumour chemotherapy, tumour vasculature.
- It must be recognized specifically and selectively by the target cells and must maintain the avidity and specificity of the surface ligands.
- The linkage of the drug and the directing unit (ligand) should be stable in plasma, interstitial and other bio-fluids.
- Carrier should be non-toxic, non-immunogenic and biodegradable particulate or macromolecule and after recognition, and internalization, the carrier system shoud release the drug moiety inside the target organs, tissues or cells.
- The bio-modules used for carrier navigation and site recognition should not be ubiquitous otherwise it may cross over the sites, defeating the concept of targeting.[6]

LEVELS OF DRUG TARGETING[6]

The various approaches of vectoring the drug to the target site can be broadly classified as:

- Passive targeting
- Inverse targeting
- Active targeting (Ligand mediated targeting and Physical targeting).
- Dual targeting
- Double targeting
- Combination targeting

PASSIVE TARGETING[6]

Systems that target the systemic circulation are generally characterized as "passive" delivery systems (i.e. targeting occurs because of the body's natural response to the physicochemical characteristics of the drug or drug-carrier system. It is a sort of passive process that utilizes the natural course of (attributed to inherent characteristics) bio-distribution of the carrier system, through which, it eventually accumulate in the organ compartment(s) of body.

The ability of some colloids to be taken up by the RES especially in liver and spleen has made them as ideal vectors for passive targeting of drugs to these compartments. Passive capture of colloidal carriers by macrophage offers therapeutic opportunities for the delivery of anti-infectives for disease conditions that involve macrophage cells of the reticuloendothelial system (RES) e.g., leishmaniasis, brucellosis and candidiasis. This category of targetable devices includes drug bearing bilayer vesicular systems as well as circular carriers of micron or sub-micron size.

The passive targetabiity of microparticulate drug carriers is due to the recognition of these particulates either in the intact or in the opsonized form, by the phagocytic cells of the RES and this sensing behavior is exploited to target MPS associated disease cell lines.

INVERSE TARGETING[6]

It is essentially based on successful attempts to avoid passive uptake of colloidal carriers by reticulo-endothelial system (RES). This effectively leads to the reversion of bio-distribution trend of the carrier and hence, the process is referred to as "Inverse targeting".

One strategy applied to achieve inverse targeting is to suppress the function of RES by a pre-injection of a large amount of blank colloidal carriers or macromolecules like dextran-sulphate. This approach leads to saturation of RES and suppression of defense mechanism. This type of targeting is an effective approach to target drug(s) to non-RES organs.

ACTIVE TARGETING [120]

In this approach carrier system bearing drug reaches to specific site on the basis of modification made on its surface rather than natural uptake by RES. Surface modification technique include coating of surface with either a bio-adhesive, nonionic surfactant or specific cell or tissue antibodies (i.e. monoclonal antibodies) or by albumin protein.

First order targeting: It involves distribution of drug carrier system to capillary bed of target site or organ. For example lymphatics, peritoneal cavity, pleural cavity, cerebral ventricles, etc.

Second order targeting: it involves delivery of drug to special cells such as tumor cells or kupffer cells in liver.

Third order targeting: Third order targeting means intracellular localization of carrier bearing drug by the process of endocytosis or via receptor based ligand mediated entry of drug carrier system, where lysosomal degradation of drug complex causes release of drug or gene delivery to nucleolus.

Ligand mediated targeting:- All the drug carrier system can become functional when they are attached with biologically relevant molecular ligand including antibodies, polypeptides, oligosaccharides, viral proteins and fusogenic residues. These types of engineered carrier selectively make the drug available to the cell or group of cells generally referred as target. In ligand mediated active targeting, reaction of a ligand to corresponding receptor enhances the

uptake of the entire drug delivery system into the cell. An example of this approach is folate receptor targeting.

Physical Targeting:-In this type of targeting some characteristics of environment changes like pH, temperature, light intensity, electric field, ionic strength, small and even specific stimuli like glucose concentration are used to localize the drug carrier to predetermined site. This approach was found exceptional for tumor targeting as well as cytosolic delivery of entrapped drug or genetic material.

DUAL TARGETING[6]

This type of targeting employs carrier molecules, which have their own intrinsic anti-viral effect thus synergies the antiviral effect of the loaded active drug. Based on this approach, drug conjugates can be prepared with fortified activity profile against the viral replication. A major advantage is that the virus replication process can be attacked at multiple points, excluding the possibilities of resistant viral strain development.

DOUBLE TARGETING[6]

In this type, if spatial targeting is combined with temporal control release results in an improved therapeutic index by the following effects;

- If drug release or activation is occurred locally at therapeutic sites, selectivity is increased by multiplication of the spatial selectivity with the local release/activation.
- The improvement in the therapeutic index by a combination of a spatially selective delivery and a preferable release pattern for a drug, such as zero order release for a longer time period of the drugs.

COMBINATION TARGETING[6]

These targeting systems are equipped with carriers, polymers and homing devices of molecular specificity that could provide a direct approach to target site. Modification of proteins and peptides with natural polymers may alter their physical characteristics and favor targeting the specific compartments, organs or their tissues within the vasculature.

VESICULAR DRUG DELI VERY SYSTEMS [7]

The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayers formed, when certain amphiphilic building blocks are confronted with water. Vesicles can be formed from a diverse range of amphiphilic building blocks. Biologic origin of these vesicles was first reported in 1965 by 'Bingham' and has been given the name Bingham bodies.

Now a days, vesicles as a carrier system have become the vehicle of choice in drug delivery and lipid vesicles were found to be of value in immunology, membrane biology and diagnostic technique and most recently in genetic engineering. Vesicular delivery system provides an efficient method for delivery to the site of infection, leading to reduction of drug toxicity with no adverse effects. Vesicular drug delivery reduces the cost of therapy by improved bioavailability of medication, especially in case of poorly soluble drugs. They can incorporate both by hydrophilic and lipophilic drugs. Different novel approaches used for delivering the drugs by vesicular system include liposomes, niosomes, sphingosomes, transferosomes and pharmacosomes.

Encapsulation of a drug in vesicular structures can be predicted to prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity, if selective uptake can be achieved. Lipid vesicles are one type of many experimental models of bio-membranes which evolved successfully, as vehicles for controlled delivery. For the treatment of intracellular infections, conventional chemotherapy is not effective, due to limited permeation of drugs into cells. This can overcome by the use of vesicular drug delivery systems.[7]

ADVANTAGES OF VESICULAR DRUG DELIVERY[7]

1. Prolong the existence of the drug in systemic circulation, and perhaps, reduces the toxicity if selective uptake can be achieved due to the delivery of drug directly to the site of infection.
2. Improves the bioavailability especially in the case of poorly soluble drugs.
3. Both hydrophilic and lipophilic drugs can be incorporated.
4. Delays elimination of rapidly metabolizable drugs and thus function as sustained release systems.[7]

These vesicular systems are accompanied with some problems like drug carriers and externally triggered (eg., temperature, pH, or magnetic sensitive) carriers load drugs passively, which may lead to low drug loading efficiency and drug leakage in preparation, preservation and transport *in- vivo.*[7]Need of intensive sonication, lead to leakages of drug during storage. Thus the major problem of their stability acts as a barrier and thus limiting their use.[8]

The targeted vesicles are classified on the basis of their composition as[9]

- Lipoidal bio-carriers.
  - Liposomes
  - Emulsomes
  - Enzymosomes
  - Ethosomes
  - Sphingosomes
  - Transferosomes
  - Pharmacosomes
  - Virosomes
- Non- lipoidal biocarriers

i. Niosomes
ii. Bilosomes
iii. Aquasomes

LIPOSOMES[10]

Liposomes or lipid based vesicles are microscopic (unilamellar or multilamellar) vesicles that are formed as a result of self-assembly of phospholipids in an aqueous media resulting in closed bi-layered structures. The assembly into closed bi-layered structures is a spontaneous process and usually needs some input of energy in the form of physical agitation, sonication, heat etc. Since lipid bi-layered membrane encloses an aqueous core, both water and lipid soluble drugs can be successfully entrapped into the liposomes. The lipid soluble or lipophilic drugs get entrapped within the bi-layered membrane whereas water soluble or hydrophilic drugs get entrapped in the central aqueous core of the vesicles.[7] For drug delivery applications, liposomes are usually uni-lamellar and range in diameter from about 50 – 150 nm.

Liposomes can be used for both oral as well as topical drug targeting. They act by the following mechanisms. They attach to cellular membrane and appear to fuse with them, releasing their content into the cell. Sometimes, they are taken up by the cell and their phospolipids are incorporated into the cell membrane by which the drug trapped inside is released.[9]

EMULSOME[11]

Emulsomes are a new generation of colloidal carrier systems in which internal core is made of fats and triglycerides which is stabilized by high concentration of lecithin in the form of o/w emulsion. Emulsomes have the characteristics of both liposomes and emulsions. The solidified or semi-solidified internal oil core provides a better opportunity to load lipophilic drugs in high concentrations. Simultaneously, a prolonged controlled release can also be expected and the ability to encapsulate water-soluble medicaments in the aqueous compartments of surrounding phospholipid layers. The solvent free and surfactant free emulsomes technology have demonstrated high drug en-capsulation capacity for water insoluble antifungal and anticancer drugs showing enhanced drug delivery and improved preclinical efficacy for parenteral routes.

ENZYMOSOME[10]

The structure in which an enzyme is enclosed in a liposome is called an enzymosome. These are liposomal constructs engineered to provide a mini bio-environment in which enzymes are covalently immobilized or coupled to the surface of the liposomes thereby targeting the drugs to the tumor cells.

ETHOSOMES[10]

Ethosomes are non-invasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are composed mainly of phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidic acid), high concentration of ethanol and water. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization therefore, when integrated into a vesicle membrane it gives that vesicle the ability to penetrate the stratum corneum. Also because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has

equivalent stability, allowing a more malleable structure and improves drug distribution ability in stratum corneum.

SPHINGOSOMES[10]

Sphingosomes are concentric, bi-layered vesicle in which an aqueous volume is enclosed by a membranous lipid bilayer mainly composed of natural or synthetic sphingolipids. Sphingosomes are comprised of sphingolipids and cholesterol, an interior aqueous environment having pH less than that of exterior. The drug is encapsulated inside the lipid bilayer and is delivered to the host at a predetermined rate thereby improving the efficacy, increasing the circulation time and reducing the toxicity. Sphingosomes can be utilized for therapeutic, cosmetic and diagnostic purpose for the delivery of active to the target site or organ. They can be administered by variety of routes like oral, parenteral, inhalation, transdermal etc. Sphingolipid present in the sphingosomes offer several advantages to these vesicular systems for targeting both by passive and active targeting mechanism.

TRANSFEROSOMES[10]

Transferosomes are specially optimized, ultradeformable (ultraflexible) lipid supra-molecular aggregates, which are able to penetrate the mammalian skin intact. Each transferosome consists of at least one inner aqueous compartment, which is surrounded by a lipid bilayer with specially tailored properties, due to the incorporation of **"edge activators"** into the vesicular membrane. Surfactants such as sodium cholate, sodium deoxy-cholate, Span 80 and Tween 80, have been used as edge activators. It was suggested that transferosomes could respond to external stress by rapid shape transformations requiring low energy. These novel carriers are applied in the form of semi-dilute suspension, without occlusion. Due to their deformability, transferosomes are good candidates for the non-invasive delivery of small, medium and large sized drugs. They have been used as drug carriers for a range of small molecules, peptides, proteins and vaccines, both *in- vitro* and *in- vivo.*

Materials commonly used for the preparation of transferosomes are phospholipids (soya phosphatidyl choline, egg phosphatidyl choline), surfactant (tween 80, sodium cholate) for providing flexibility, alcohol (ethanol, methanol) as a solvent, dye for confocal scanning laser microscopy (CSLM) and buffering agent (saline phosphate buffer pH 7.4), as a hydrating medium.

PHARMACOSOMES[7]

These are defined as colloidal dispersions of drugs covalently bound to lipids and may exist as ultrafine vesicular, micellar or hexagonal aggregates, depending on the chemical structure of drug-lipid complex. The pro-drug conjoins hydrophilic and lipophilic properties and therefore acquires amphiphilic characters and was found to reduce interfacial tension and thus at higher concentrations exhibits mesomorphic behavior. Because the system is formed by linking a drug (pharmakon) to a carrier (soma), they are called pharmacosomes. Pharmacosomes bearing unique advantages over liposome and niosome vesicles have come up as potential alternative to conventional vesicles.

VIROSOMES[11]

Virosomes are reconstituted viral envelopes including membrane lipids and viral spike glycoproteins but devoid of viral genetic material. The external surface of the virosome resembles that of a virus particle with spike proteins protruding from the membrane but their interior compartment is empty. Virosomes were first prepared with inserted purified influenza spike proteins into preformed liposomes. Thereafter a range of viral envelopes have been reconstituted including those of Sendai virus, Semliki Forest Virus (SFV), Vesicular Stomatitis Virus (VSV) and Sindbis virus. Because virosomes display viral envelope glycoproteins which in their native conformation stimulate humoral responses, they are highly effective as vaccine antigens and adjuvants.

NIOSOMES[7]

Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle. The vesicle is composed of a bilayer of non-ionic surface active agents and hence the name niosomes. The niosomes are very small, and microscopic in size. Their size lies in the nano-metric scale. Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs. Although structurally similar to liposomes, they offer several advantages over them.

COLLOIDOSOMES[10]

Colloidosomes are the hollow shell microcapsules consisting of coagulated or fused particles at interface of emulsion droplets. Colloidosomes have exciting potential applications in controlled release of drugs, proteins, vitamins as well as in cosmetics and food supplements. Colloidosomes have a great encapsulation efficacy with a wide control over size, permeability, mechanical strength and compatibility. Colloidosomes assemble polymer latex, colloidal particles into shells around water-in-oil emulsion drops followed by partial fusion of shell and centrifugal transfer into water to yield stable capsules in which the shell permeability can be controlled by adjustment of partial fusion conditions. Hairy colloidosomes whose shell consists of micro-rod particles, are designed and fabricated novel colloidosome capsules that consist of aqueous gel core and shells of polymeric micro-rods. This has been achieved by templating water-in-oil emulsions stabilized by rod like particles followed by gelling of the aqueous phase, dissolution of oil phase in ethanol and re-dispersion of obtained colloidosome microcapsules in water.

HERBOSOMES[10]

The term "herbo" means plant, while "some" means cell-like. Over the past century, phyto- chemical and phyto-pharmacological sciences established the compositions, biological activities and health promoting benefits of numerous botanical products. Most of the biologically active constituents of plants are polar or water soluble molecules. However, water soluble phyto-constituents (like flavonoids, tannins, glycosidic aglycones etc) are poorly absorbed either due to their large moleculer size which cannot be absorbed by passive diffusion, or due to their poor lipid solubility, severely limiting their ability to pass across the lipid rich biological membranes, resulting in poor bioavailability. Phyto-medicines, complex chemical mixtures prepared from plants, have been used for health maintenance since ancient times. But many phyto-medicines are limited in their effectiveness because they are poorly absorbed when taken by mouth. Herbosomes are also often known as phyto-somes. Molecular layer consisting of PC and other phospholipids provides a continuous matrix into which the proteins insert.

CUBOSOMES[10]

Bicontinuous cubic liquid crystalline materials are active ingredients because they give the unique structural ends to controlled release applications. Amphiphilic molecules form bi-continuous water and oil channels, where "bi-continuous" refers to two distinct (continuous, but non-intersecting) hydrophilic regions separated by the bilayer. Cubosomes are discrete, sub micron, nanostructured particles of bicontinuous cubic liquid crystalline phase. Cubosomes possess the same microstructure as the parent cubic phase but have much larger specific surface area and their dispersions have much lower viscosity than the bulk cubic phase. The ability of cubic phases to exist as discrete dispersed colloidal particles or cubosomes is perhaps the most intriguing. Whereas most concentrated surfactants that form cubic liquid crystals lose these phases to micelle formation at high dilutions, a few surfactants have optimal water insolubility. Their cubic phases exist in equilibrium with excess water and can be dispersed to form cubosomes.

LAYEROSOMES[10]

The layer-by-layer coating concept is one of the strategies used for the preparation or the stabilization of nanosystems. The layersomes are conventional liposomes coated with one or multiple layers of biocompatible polyelectrolytes in order to stabilize their structure. The formulation strategy is based on an alternative coating procedure of positive poly (lysine) (pLL) and negative poly (glutamic acid) (pGA) polypeptides on initially charged small uni-lamellar liposomes. Oral administration or their incorporation in biomaterials is among potential fields of application.

UFASOMES[10]

The fatty acid vesicles are named "ufasomes," ufosomes are unsaturated fatty acid liposomes. Fatty acid vesicles are colloidal suspensions of closed lipid bilayers that are composed of fatty acids and their ionized species (soap). They are observed in a small region within the fatty acid-soap-water ternary phase diagram above the chain melting temperature (Tm) of the corresponding fatty acid-soap mixture. Fatty acid vesicles always contain two types of amphiphiles, the non-ionized neutral form and the ionized form (the negatively charged soap). The ratio of non- ionized neutral form and the ionized form is critical for the

vesicle stability. Fatty acid vesicles are actually mixed "fatty acid/soap vesicles". Ufasome membranes are much more stabilized in comparison to liposomes.

CRYPTOSOMES[11]

Stealth liposomes, also known as immune-liposomes or cryptosomes are liposomes that evade detection in an immune system. Stealth liposomes or cryptosomes are designed to circulate for longer periods of time *in- vivo*, but they are different from "long-circulating liposomes". Stealth liposomes use polyethylene glycol (PEG) as a steric stabilizer. The properties of the stealth liposome depend on the way PEG is linked to the lipids. It is important to note that stealth liposomes are not fully inert vesicles; they can eventually become detected by the immune system. It could be used for slow release of drug or for imaging purposes. Incorporation of polymers, such as polyethylene glycol-lipid derivatives, or glycolipids into liposomes results in sterically stabilized liposomes which have several advantages over liposome formulations traditionally used in the past, including reduced recognition and uptake by macrophages, extended circulation half-lives, targeted drug delivery dose-independent pharmacokinetics, and increased uptake *in- vivo* by solid tumors, breast cancer.

ULTRASOMES[11]

Ultrasomes are specialized liposomes encapsulating an endonuclease enzyme extracted from *Micrococcus luteus*. Endonuclease recognizes ultra violet (UV) damage and is reported to accelerate its repair four-folds. Ultrasomes also protect the immune system by repairing UV-DNA damage and reducing the expression of tumor necrosis factor (TNF-α), interleukins (IL-1, IL-6 and IL-8). They stimulate the production of melanin by melanocytes in the tanning response following UV exposure and are used in cosmeceuticals and anti-aging formulations.

PHOTOSOMES[11]

These are artificial spherical submicroscopic vesicles with diameter between 25 and 5000 nm. Photosomes are composed of amphiphilic molecules with core that consists of an aqueous cavity, which is encapsulated by one or more bimolecular phospholipid sheets separated from each other by aqueous layers. Photosomes contain the enzyme photolysase encapsulated in a liposome structure and are incorporated in sun-care product to protect the sun exposed skin by releasing a photo-reactivating enzyme extracted from a marine plant,

Anacystis nidulans. This enzyme can be activated by light and can work during the day to support the skin deoxyribonucleic acid (DNA) repair process.

Combined with ultrasomes, they constitute the "intelligent" DNA repair system and are the most widely used in cosmetic delivery systems and photodynamic therapy.

GENOSOMES[11]

Genosomes are complex of genetic material like DNA and suitable lipid. They are also known as lipoplexes that are used to deliver genes. They are artificial macromolecular complexes for functional gene transfer. Cationic lipids are most suitable for this delivery system because they possess high biodegradability and stability in the blood stream. Mostly DNA-cationic liposome complexes were used to translocate DNA across cellular membranes *in- vivo*, because interaction between DNA-lipid membranes has proved crucial to the understanding of the colloidal state of the genosomes. These DNA lipid complexes could be later aggregated into higher order assemblies, creating stacked lipid-DNA multilayers, for generating more protection.

VESOSOME[11]

Vesosomes are multi-compartment structures which has distinct inner compartments separated from the external membrane. Each compartment of vesosome can encapsulate different materials and have different bilayer composition. Vesosome could entrap both colloidal particles and bio-logical macromolecules relatively efficiently. While small molecules are released from uni-lamellar liposomes in minutes, they are retained in vesosomes from hours to days, even though the liposomes and vesosomes have the same bilayer composition and size. Vesosomes are formed by adding ethanol to a variety of saturated phospholipids. At temperatures below the gel-liquid crystalline transition, Tm, the inter digitated lipid-ethanol sheets are rigid and flat; when the temperature is raised above Tm, the sheets become flexible and close on themselves and the surrounding solution to form closed compartments.

SUBTILOSOMES[11]

Subtilosomes are prepared from phospholipids isolated from *Bacillus subtilis*. They are novel potential carrier system used in drug delivery. Cardiolipin and phosphatidyl glycerol are abundant in *B. subtilis*.

ESCHERIOSOMES[11]

These are lipoidal vesicles, prepared from polar lipids extracted from *Escherichia coli.* Majorly phosphatidyl ethanolamine, cardiolipin, and phosphatidyl glycerol are classes of phospholipid, present in *E. coli.* Escheriosomes encapsulated antigen elicit strong humoral immune response in immunized animals, in general, escheriosomes are considered as potential candidate vaccine carrier system capable of eliciting both cell-mediated as well as humoral immune responses.

MARINOSOMES[11]

Marinosomes are liposomes based on a natural marine lipid extract containing high ratio of polyunsaturated fatty acids (PUFA) like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are not present in normal skin epidermis. They are metabolized by skin epidermal enzymes into anti-inflammatory and anti-proliferative metabolites that are beneficial in treating inflammatory skin disorders. However, the preventing effect of marinosomes was highly dependent on the lipid concentration used and the liposome mean diameter. Active and passive loading of drug, as well as complex structural rearrangements directly depends on transmembrane pH gradient. All these results allowed considering marinosomes as potential candidates for cosmeceutical and oral PUFA supplements in view of the prevention and treatment of deficiencies.

ASPASOMES[12]

Vesicles prepared with amphiphiles having antioxidant property may have potential applications towards disorders implicated with reactive oxygen species. Ascorbyl palmitate (ASP) was explored as bi-layered vesicle forming material. It forms vesicles in combination with cholesterol and a negatively charged lipid (dicetyl phosphate). Aspasome rendered much better antioxidant activity.

ARCHAEOSOME[11]

Archaeosome constitute a novel family of liposome made with one or more of the fully saturated bipolar tetra ether lipids, which exerts a higher stability in comparison with conventional lipids to several conditions such as high temperature, alkaline or acidic pH, and presence of phospholipases, bile salts and serum media. They are nano-sized vesicles prepared from total polar lipids either extracted from the selected genera and species of the Archaea domain or synthetic archaeal lipids. Incorporation of polyethylene glycol and coenzyme Q10 into archaeosomes has been found to alter the tissue distribution profiles of intravenously administered vesicles. Also, intravenous and oral delivery of nanometric-sized archaeosomes to an animal model was well tolerated with no apparent toxicity.

DISCOMES[13]

Niosomes are solubilised with Sollulan C24 (polyoxy ethylene cetyl ether class) in order to effect vesicle to discome transition. Discomes are relatively large in size (12- 60 micrometer) and are capable of entrapping high quantities of water- soluble solutes. They were found to release the contents following biphasic profile particularly in the case where the drug was loaded using pH gradient technique. The prepared system could produce or sustain a suitable activity profile upon administration. The discomes were found to be promising and of potential for controlled ocular administration of water- soluble drugs.

BILOSOMES[9]

Bilosomes are the novel innovative drug delivery carriers consist of deoxycholic acid incorporated into the membrane of niosomes. As conventional vesicles (liposomes and niosomes) can cause dissolution and undergo enzymatic degradation in gastro-intestinal tract but incorporation of bile salts (commonly used penetration enhancers) in niosomal formulation could stabilize the membrane against the detrimental effects of bile acids in GI tract. These bile salt stabilized vesicles are known as bilosomes. These are highly biocompatible and have been found to improve the therapeutic efficacy of drugs due to their stability in gastro intestinal tract. Bilosomes have been found to increase the bio-availability of drugs as they can readily absorbed through small intestine to the portal circulation (hepato-circulation). Through this circulation they approach to liver and release the drug, so found to be an effective tool in drug targeting to liver.

AQUASOMES[9]

Aquasomes are three layered structures (i.e. core, coating and drug) that are self-assembled through non covalent bonds, ionic bonds and vander-waals forces. They consist of tin oxide, nano-crystalline carbon ceramic (diamonds) or brushite (calcium phosphate dihydrate) core coated with oligomeric film to which biochemically active molecules are adsorbed by copolymerization, diffusion or adsorption with or without modification. The solid core provides the structural stability, while the carbohydrate coating protects against dehydration and stabilizes the biochemically active molecules. Aquasomes are spherical, 60-300nm size particles called "bodies of water". Due to their size and structural stability, these avoid clearance by reticuloendothelial system and degradation by other environmental changes.

HEMOSOMES[14]

New artificial oxygen transporting systems engineered by immobilizing haemoglobin with polymerisable phospholipids, capable of prolonged activity in the circulation are of special interest. Natural hemoglobin was incorporated into liposomes of different composition (so- called hemosome). It was shown that the maximal quantity of hemoglobin obtained from lysed erythrocytes incorporates into negatively charged liposomes. Stabilized hemosomes bind oxygen in the same way as human blood hemolysates.

NIOSOMES

INTRODUCTION

Vesicles are colloidal particles in which a concentric bilayer made-up of amphiphilic molecules surrounds an aqueous compartment. They are a useful vehicle for drug delivery of both hydrophobic drugs, which associate with the lipid bilayer and hydrophilic drugs, which are encapsulated in the interior aqueous compartment. In general, vesicles made of natural or synthetic phospholipids are called liposomes whereas those made of nonionic surfactants (e.g. alkyl ethers and alkyl esters) and cholesterol constitutes a nonionic surfactant vesicular system called niosomes.[15]

Liposomes are colloidal, concentric bi-layered vesicles where aqueous compartment is entirely enclosed by a bilayer membrane, mainly composed of natural or synthetic lipids.[16]

Disadvantages of Liposomes as Vesicular Drug Delivery System

- Liposomes are leaky in nature leading to premature drug release.
- Poor encapsulation efficiency for hydrophilic drug.
- Liposomes are expensive.
- Liposomes possess short half-life.

Requirement of cryogenic atmosphere for the handling of liposomes have prompted the use of nonionic surfactants for the preparation of vesicular drug delivery systems. This newly introduced vesicular drug delivery system was termed as niosome which consist of unilamellar or multilamellar vesicles.

Niosomes, that is, non-ionic surfactant vesicles, are microscopic lamellar vesicles formed when nonionic surfactants (mainly of alkyl or dialkyl polyglycerol ether class) are added to cholesterol with subsequent hydration in aqueous media. Addition of cholesterol provides rigidity to the bilayer leading to the formation of less permeable niosomes. Addition of nonionic surfactants to niosomes increases the size of vesicles and provides charge to the vesicles and hence increases the entrapment efficiency of niosomes. Niosomes possess structure that is similar to liposomes and hence represent a promising drug delivery module.

Niosomes are expected to be better drug carrier system than liposomes upon consideration of factors like cost, stability, entrapment efficiency, bioavailability, and so forth.[16]

Advantages[17]

- They improve the therapeutic performance of the drug by protecting it from biological environment and restricting effects to target cells thereby reducing the clearance of the drug.
- They help to accommodate hydrophilic, hydrophobic as well as ampiphilic drug moieties, so they can be used for variety of drugs.
- Niosomes offer a controlled release of drug.
- They can increase the oral bio-availability of drugs.
- They are osmotically active and stable.
- They increase the stability of the entrapped drug.
- They are flexible and easily modulated.
- They can enhance the skin penetration of drugs.
- They can be made to reach the target site by oral, parenteral as well as topical routes.
- The surfactants are biodegradable, biocompatible, and non-immunogenic.
- Handling and storage of surfactants are very easy.

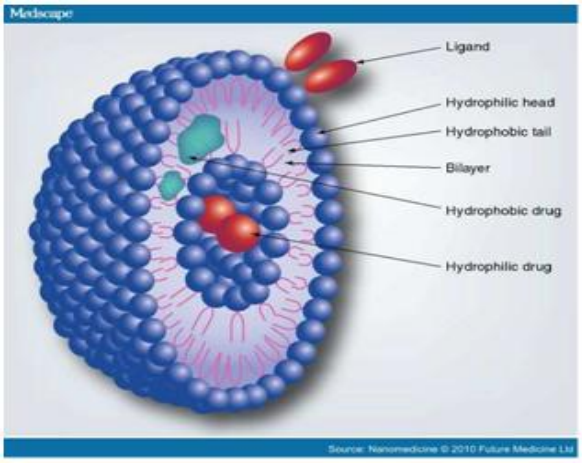
Limitation of niosomal drug delivery system[17]

Though niosomal formulation has number of advantages in NDDS system there are a few factors that limit its usage.

- The aqueous suspension of niosomes may have limited shelf life due to fusion, aggregation, leaking of entrapped drugs and hydrolysis of encapsulated drug.
- The preparations of multi-lamellar vesicles such as sonication, extrusion are time consuming and required special instruments.

Structure of niosomes[18]

Fig.2.



Structural components of Niosomes

1. **Surfactants:**

A wide range of surfactants and their combinations in different molar ratios have been used to entrap many drugs in niosomes of varying features such as size.

Non- ionic surfactants are the most common type of surface active agent used in preparing vesicles due to the superior benefits they impart with respect to stability, compatibility and toxicity compared to their anionic, amphoteric or cationic counterparts. They are generally less toxic, less hemolytic and less irritating to cellular surfaces and tend to maintain near physiological pH in solution. They are also strong P- glycoprotein inhibitors, a property useful for enhancing drug absorption and for targeting to specific tissues.[15]

The various types of non- ionic surfactants are[19]

a. **Ether linked surfactants:**

These are polyoxyethylene alkyl ethers which have hydrophilic and hydrophobic moieties linked with ether. The general formula of this group is CnEOm, where n can be 12-18 and m can be 3-7. Surfactants with polyhydroxyl head and ethylene oxide units are also reported to be used in niosomes formation. Single alkyl chain surfactant C16 mono

alkyl glycerol ether with an average of three glycerol units is one of the examples of this class of surfactants used for the preparation of niosomes. Polyoxyethylene cetyl ethers (Brij58) and Polyoxyethylene stearyl ethers (Brij72 and 76) are also used in preparation of niosomes.

b. **Ester linked surfactants:**

These surfactants have ester linkage between hydrophilic and hydrophobic groups and have been studied for its use in the preparation and delivery of sodium stilbogluconate to the experimental marine visceral leishmaniasis.

c. **Sorbitan Esters:**

These are most widely used ester linked surfactants especially in food industry. The commercial sorbitan esters are mixtures of the partial esters of sorbital and its mono and di-anhydrides with oleic acid. These have been used to entrap wide range of drugs viz doxorubicin.

d. **Alkyl Amides:**

These are alkyl galactosides and glucosides which have incorporated amino acid spacers. The alkyl groups are fully or partially saturated C_{12} to C_{22} hydrocarbons and some novel amide compounds have fluorocarbon chains.

e. **Fatty Acids and Amino Acid Compounds:**

These are amino acids which are made amphiphilic by addition of hydrophobic alkyl side chains and long chain fatty acids which form "Ufasomes" vesicles formed from fatty acid bilayers.

2. Cholesterol:[19]

Steroids bring about changes in fluidity and permeability of the bilayer and are thus important components. Cholesterol a waxy steroid metabolite is usually added to the non-ionic surfactants to provide rigidity and orientational order. It does not form the bilayer itself and can be incorporated in large molar ratios.

Cholesterol is an amphiphilic molecule; it orients its -OH group towards aqueous phase and aliphatic chain towards surfactant's hydrocarbon chain. Rigidization is provided by alternative positioning of rigid steroidal skeleton with surfactant molecules in the

bilayer by restricting the movement of carbons of hydrocarbon. Cholesterol is also known to prevent leakage by abolishing gel to liquid phase transition

3. **Charge Inducers:**[19]

Charge inducers increase the stability of the vesicles by induction of charge on the surface of the prepared vesicles. It act by preventing the fusion of vesicles due to repulsive forces of the same charge and provide higher values of zeta potential.

The negative charge inducers are dicetyl phosphate, dihexadecyl phosphate and lipoamine acid.

The positive charge inducers are sterylamine and cetyl pyridinium chloride.

Types of niosomes[20]

The niosomes are classified on the basis of

- The number of bilayer (e.g. MLV, SUV)
- Size (e.g. LUV, SUV)
- The method of preparation (e.g. REV, DRV).

The various types of niosomes are described below:

1. **Multilamellar vesicles (MLV):**

It consists of a number of bilayer surrounding the aqueous lipid compartment separately. The approximate size of these vesicles is 0.5-10 μm diameter. Multilamellar vesicles are the most widely used niosomes. It is simple to make and are mechanically stable upon storage for long periods. These vesicles are highly suited as drug carrier for lipophilic compounds.

2. **Large unilamellar vesicles (LUV):**

Niosomes of this type have a high aqueous/lipid compartment ratio, so that larger volumes of bio-active materials can be entrapped with a very economical use of membrane lipids.

3. **Small unilamellar vesicles (SUV):**

These small unilamellar vesicles are mostly prepared from multilamellar vesicles by sonication method, French press extrusion, electrostatic stabilization is the inclusion of dicetyl phosphate in 5(6)- carboxyfluorescein (CF) loaded Span 60 based niosomes.

METHODS OF PREPARATION[21]

A. Hand shaking method (Thin film hydration technique)

The mixture of vesicles forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethyl ether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature (20°C) using rotary evaporator leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film can be rehydrated with aqueous phase at 0-60°C with gentle agitation. This process forms typical multilamellar niosomes.

B. Micro fluidization

Micro fluidization is a recent technique used to prepare unilamellar vesicles of defined size distribution. This method is based on submerged jet principle in which two fluidized streams interact at ultra high velocities, in precisely defined micro channels within the interaction chamber. The impingement of thin liquid sheet along a common front is arranged such that the energy supplied to the system remains within the area of niosomes formation. The result is a greater uniformity, smaller size and better reproducibility of niosomes formed**.**

C. Reverse Phase Evaporation Technique (REV)

Cholesterol and surfactant (1:1) are dissolved in a mixture of ether and chloroform. An aqueous phase containing drug is added to this and the resulting two phases are sonicated at 4 5°C. The clear gel formed is further sonicated after the addition of a small amount of phosphate buffered saline (PBS). The organic phase is removed at40°C under low pressure. The resulting viscous niosome suspension is diluted with PBS and heated on a water bath at 60°C for 10min to yield niosomes.

D. Ether injection method

This method provides a means of making niosomes by slowly introducing a solution of surfactant dissolved in diethyl ether into warm water maintained at 60°C. The surfactant mixture in ether is injected through 14-gauge needle into an aqueous solution of material. Vaporization of ether leads to formation of single layered vesicles. Depending upon the conditions used, the diameter of the vesicle range from 50 to1000 nm.

E. Trans membrane pH gradient (inside acidic) Drug Uptake Process (remote Loading)

Surfactant and cholesterol are dissolved in chloroform. The solvent is then evaporated under reduced pressure to get a thin film on the wall of the round bottom flask. The film is hydrated with citric acid (pH4.0) by vortex mixing. The multilamellar vesicles are frozen and thawed 3 times and later sonicated. To this niosomal suspension, aqueous solution containing 10 mg/ml of drug is added and vortexed. The pH of the sample is then raised to 7.0-7.2 with 1M disodium phosphate. This mixture is later heated at 60°C for 10 minutes to give niosomes.

F. The "Bubble" Method

It is novel technique for the one step preparation of liposomes and niosomes without the use of organic solvents. The bubbling unit consists of round-bottomed flask with three necks positioned in water bath to control the temperature. Water-cooled reflux and thermometer is positioned in the first and second neck and nitrogen supply through the third neck. Cholesterol and surfactant are dispersed together in this buffer (pH 7.4) at 70°C, the dispersion mixed for 15 seconds with high shear homogenizer and immediately afterwards "bubbled" at 70°C using nitrogen gas.

G. Sonication

A typical method of production of the vesicles is by sonication of solution as described by Cable. In this method an aliquot of drug solution in buffer is added to the surfactant/ cholesterol mixture in a 10-ml glass vial. The mixture is probe sonicated at 60°C for 3 minutes using a sonicator with a titanium probe to yield niosomes.

H. Formation of niosomes from proniosomes

Another method of producing niosomes is to coat a water-soluble carrier such as sorbitol with surfactant. The result of the coating process is a dry formulation, in which

each water-soluble particle is covered with a thin film of dry surfactant. This preparation is termed "Proniosomes". The niosomes are recognized by the addition of aqueous phase at T > Tm and brief agitation.

T=Temperature.

Tm = mean phase transition temperature.

Blazek-Walsh A.I. *et al* .have reported the formulation of niosomes from maltodextrin based proniosomes. This provides rapid reconstitution of niosomes with minimal residual carrier. Slurry of maltodextrin and surfactant was dried to form a free flowing powder, which could be rehydrated by addition of warm water.[21]

Formulation aspects and factors affecting niosome formation

1. Surfactants[15]

The formation of bilayer vesicles instead of micelles is dependent on the hydrophilic- lipophilic balance (HLB) of the surfactant, the chemical structure of the components and the critical packing parameter (CPP).

a) Chemical structure of the surfactant[15]

The chain length and size of the hydrophilic head group of the nonionic surfactant affect the entrapment efficiency of drug. Nonionic surfactants with stearyl (C_{18}) chains show higher entrapment efficiency than those with lauryl (C_{12}) chains. The Tween series of surfactants bearing a long alkyl chain and a large hydrophilic moiety in combination with cholesterol in a 1:1 ratio have the highest entrapment efficiency of water soluble drugs.

b) HLB value and phase transition temperature of surfactant[15]

The HLB value of surfactant plays a key role in controlling drug entrapment of the vesicle.

- A surfactant with an HLB value in the range 14- 17 is not suitable to produce niosomes
- HLB value of 8.6 gives niosomes with the highest entrapment efficiency.
- Entrapment efficiency decreases as the HLB value decreases from 8.6 to 1.7
- For HLB> 6, cholesterol must be added to the surfactant in order to form a bilayered vesicle and for lower HLB value, cholesterol enhances stability of vesicles.

- The entrapment efficiency is affected by the phase transition temperature (T_c) of the surfactant. Thus Span 60 with a high T_c exhibits the highest entrapment efficiency.
- Polyoxyethelene 4- lauryl ether (Brij30) has an HLB value of 9.7, phase transition temperature <100°C and cannot be used to formulate some drugs and iodides, mercury salts, phenolic substances, salicylates, sulfonamides and tannins as it cause oxidation leading to discoloration of product.

c) Critical packing parameter (CPP)[22] and Israelachvili Hypothesis [6]

The key factor that dictates the aggregation of amphiphile molecules into bilayer vesicles rather than, for example micelles or liquid crystals, is the molecular shape of the amphiphile as this will influence their geometrical packaging within a given solution environment.

Isrealachvili suggested that parameters of self-assemblages are governed by a critical packing parameter (CPP). Their self- organization in water is mainly the result of the hydrophobic effect, as in the case of soap and detergent, however, it also depends on the molecular geometry. The symmetry of lipid self-assembly and liquid crystalline-phase formation show strong dependence on the molecular shape of the mesogen/amphipiles. The different shapes and volumes constructing different phases are characterized by a dimensionless critical packing parameter (CPP)

$$CPP = \nu/l_c A_p = A_{hp} / A_p$$

Where, ν = Hydrophobic group volume, l_c = the critical hydrophobic group length and A_p = the cross-sectional area of hydrophobic group.

A CPP below 0.5 (indicating a large contribution from the hydrophilic head group area) is reported to give spherical micelles and above 1 (indicating a large contribution from the hydrophobic group volume) should produce inverted micelles. A CPP of between 0.5 and 1 indicates that the surfactant is likely to form vesicles.

The cross sectional geometry of hydrophobic and hydrophilic domain suggests the stable geometric configuration. For $A_{hp} > A_p$, structures with high curvature (such as micelles) are formed. Then the areas are comparable $A_{hp} \approx A_p$ a bilayered configuration is the most stable form, while for $A_{hp} < A_p$, inverse micelles (with negative surface

curvature) are formed. The molecular shape analysis and the concept of shape parameters are very useful for qualitative understanding of the topology of lipid vesicles based on different lipid composiions.

2. Cholesterol

Inclusion of cholesterol in niosomes increases its hydrodynamic diameter and entrapment efficiency. In general, the action of cholesterol is two folds; on one hand, cholesterol increases the chain order of liquid-state bilayers and on the other, cholesterol decreases the chain order of gel state bilayers. At a high cholesterol concentration, the gel state is transformed to a liquid ordered phase.[23]

An increase in cholesterol content of the bilayers resulted in a decrease in the release rate of encapsulated material and therefore an increase of the rigidity of the bilayers obtained. Presence of charge tends to increase the interlamellar distance between successive bilayers in multi-lamellar vesicle structure and leads to greater overall entrapped volume.[23]

The amount of cholesterol to be added depends on the HLB value of the surfactants. As the HLB value increases above 10, it is necessary to increase the minimum amount of cholesterol to be added in order to compensate for the larger head groups. Higher entrapment of Minoxidil occurred in Brij76 niosomes in the presence of a higher content of cholesterol whereas no significant increase in entrapment efficiency occurred in Brij52 (HLB5.3) niosomes. In fact, above a certain level of cholesterol, entrapment efficiency decreased possibly due to a decrease in volume diameter ($CPP < 0.05$).[15]

3. Drug[23]

Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayers, thereby increasing vesicle size. In polyoxyethylene glycol (PEG) coated vesicles, some drug is entrapped in the long PEG chains, thus reducing the tendency to increase the size. The hydrophilic lipophilic balance of the drug also affects degree of entrapment.

4. Resistance to osmotic stress[23]

Addition of a hypertonic salt solution to a suspension of niosomes brings about reduction in diameter. In hypotonic salt solution, there is initial slow release with slight swelling of vesicles probably due to inhibition of eluting fluid from vesicles, followed by faster release, which may be due to mechanical loosening of vesicles structure under osmotic stress.

5. Membrane Composition[23]

The stable niosomes can be prepared with addition of different additives along with surfactants and drugs. Niosomes formed have a number of morphologies and their permeability and stability properties can be altered by manipulating membrane characteristics by different additives.

In case of polyhedral niosomes formed from C16G2, the shape of these polyhedral niosome remains unaffected by adding low amount of solulan C24 (cholesteryl poly-24-oxy ethylene ether), which prevents aggregation due to development of steric hindrance**.** In contrast, spherical niosomes are formed by C16G2: cholesterol: solulan (49:49:2)**.**

The mean size of niosomes is influenced by membrane composition such as Polyhedral niosomes formed by C16G2: solulan C24 in ratio (91:9) having bigger size (8.0 ± 0.03mm) than spherical/tubular niosomes formed by C16G2: cholesterol: solulan C24 in ratio (49:49:2) (6.6±0.2mm). Addition of cholesterol molecule to niosomal system provides rigidity to the membrane and reduces the leakage of drug from niosome.

6. Temperature of hydration[21]

Hydration temperature influences the shape and size of the niosome. For ideal condition it should be above the gel to liquid phase transition temperature of system. Temperature change of niosomal system affects assembly of surfactants into vesicles and also induces vesicle shape transformation.

7. pH of hydration medium[15]

Entrapment efficiency of niosomes is greatly affected by the pH of the hydration medium. High entrapment of flurbiprofen was reported at acidic pH with a maximum encapsulation efficiency of 94.6% at pH 5.5. The fraction of flurbiprofen encapsulated

increased to about 1.5 times as pH decreased from 8 to 5.5 and decreased significantly at pH> 6.8. The lowest entrapment of flurbiprofen occurred at pH 7.4 and 8 with no significant difference between them. The increase in encapsulation efficiency of flurbiprofen at lower pH is presumably due to its ionizable carboxylic acid group. At lower pH, the proportion of unionized flurbiprofen increases and partitions more readily into the lipid bilayer than the ionized species. At lower pH, niosome formulations should be examined by optical microscopy for the presence of drug precipitates both before and after centrifugation and washing. This will help to determine the concentration of drug in the hydration medium giving optimum encapsulation in niosomes.

SEPARATION OF UNENTRAPPED DRUG[21]

The removal of unentrapped solute from the vesicles can be accomplished by various techniques, which include: -

1. **DIALYSIS**

   The aqueous niosomal dispersion is dialyzed in dialysis tubing against phosphate buffer or normal saline or glucose solution.

2. **GEL FILTRATION**

   The un-entrapped drug is removed by gel filtration of niosomal dispersion through a Sephadex-G-50 column and elution with phosphate buffered saline or normal saline.

3. **CENTRIFUGATION**

   The niosomal suspension is centrifuged and the supernatant is separated. The pellet is washed and then re-suspended to obtain a niosomal suspension free from unentrapped drug.

CHARACTERIZATION OF NIOSOME[17, 19]

Size and shape: Shape of niosomal vesicle is assumed to be spherical, and various methods can be used for the determination of mean diameter like laser light scattering, electron microscopy, molecular sieve chromatography, photon correlation microscopy, optical microscopy, freeze fracture microscopy.

Bilayer formation: Assembly of non-ionic surfactants to form a bilayer vesicle is characterized by an X-cross formation under light polarization microscopy.

Number of lamellae: This is determined by using nuclear magnetic resonance (NMR) spectroscopy, small angle X-ray scattering and electron microscope.

Membrane rigidity: Membrane rigidity can be measured by means of mobility of fluorescence probe as a function of temperature.

Entrapment efficiency: After preparing niosomal dispersion, unentrapped drug is separated by dialysis, centrifugation, or gel filtration as described above and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analyzing the resultant solution by appropriate assay method for the drug.

Entrapment efficiency = (Amount entrapped / total amount) x 100.

Vesicular surface charge: Niosomes are generally prepared by the inclusion of charged molecules in bilayer to prevent the aggregation of vesicles. A reduction in aggregate formation was observed when charged molecule like dicetyl phosphate was incorporated in vesicles. The charges on vesicles are expressed in terms of zeta potential and calculated using the Henry's equation.

$$£ = \mu E \Pi \eta / \Sigma$$

where,

£ - Zeta potential

μE - Electrophoretic mobility

η - Viscosity of medium

Σ - Dielectric constant

Stability study: Stability studies are performed by storing niosome at two different conditions, usually 4±1°C and 25±2°C. Formulation size, shape and number of vesicles per cubic mm can be measured before and after storing for 30 days. After 15 and 30 days, residual drug can also be measured. Light microscope is used for determination of size of vesicles and the numbers of vesicles per cubic mm is measured by haemocytometer.

Number of niosomes per cubic mm = Total number of niosomes x dilution factor x 400/ Total number of small squares counted.

***In- vitro* release study[17]**

Dialysis: A method of *in- vitro* release rate study was reported with the help of dialysis tubing. A dialysis bag was washed and soaked in distilled water. The vesicle suspension was pipetted into a bag made up of the tubing and sealed. The bag containing the vesicles was then placed in 200 ml buffer solution in a 250 ml beaker with constant shaking at 25°C or 37°C. At various time intervals, the buffer was analyzed for the drug content by an appropriate assay method.

Reverse dialysis: In this technique, niosomes are placed in a number of small dialysis tubes containing 1ml of dissolution medium and the niosome are then displaced from the dissolution medium.

Franz diffusion cell: In the Franz diffusion cell, the cellophane membrane is used as the dialysis membrane. Niosomes are dialyzed through a cellophane membrane against a suitable dissolution medium at room temperature .The samples are withdrawn at suitable time interval and analyzed for drug content.

***In- vivo* release study:**

Albino rats are used for this study. These rats are subdivided with groups. Niosomal suspension used for *in- vivo* study is injected intravenously (through tail vein) using appropriate disposal syringe.

APPLICATIONS OF NIOSOMES

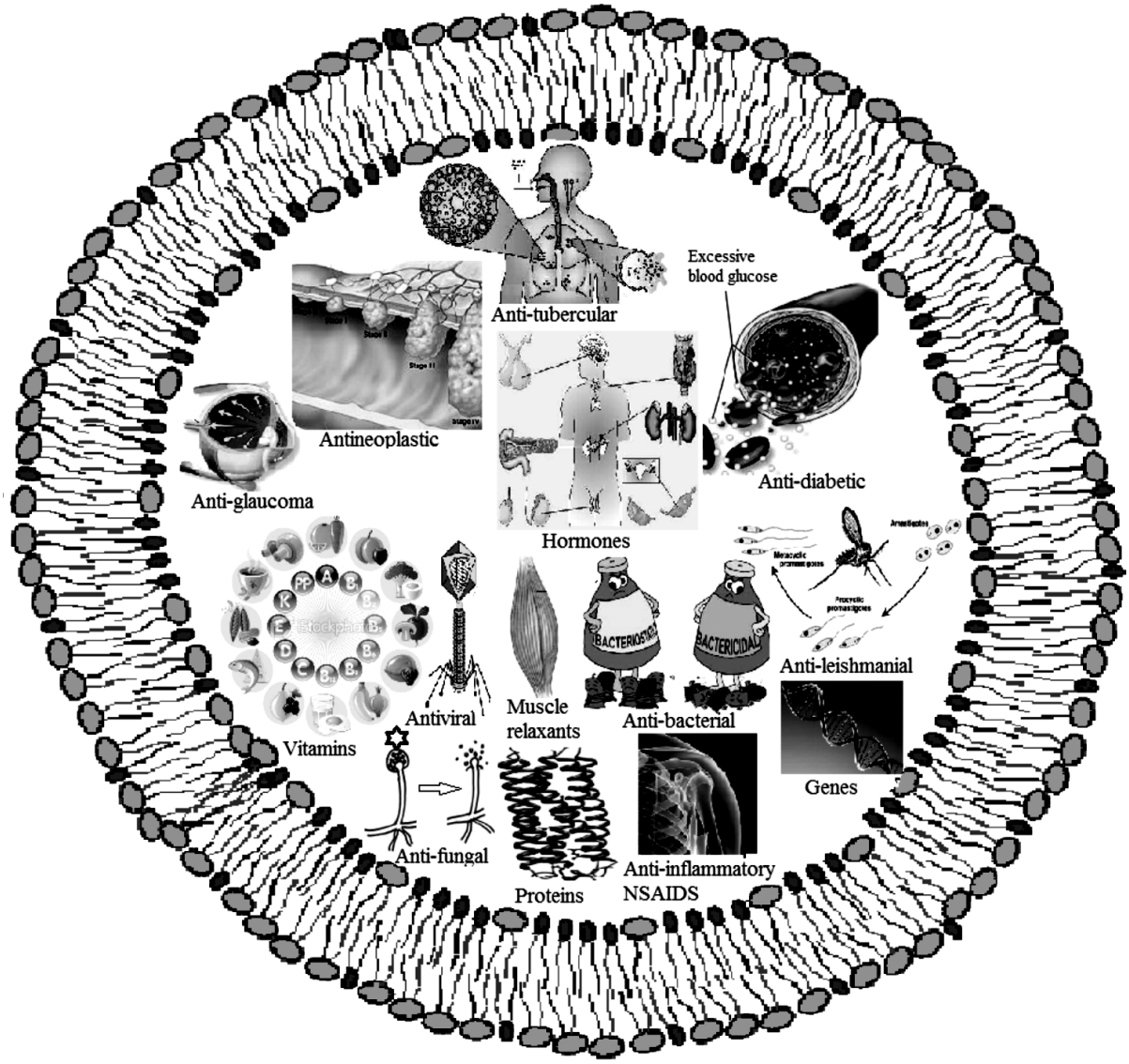


Fig. 3. Applications of niosomes.

The applications of niosomes can be mainly classified into three categories[25, 26]

1. For controlled release of drugs
2. To improve the stability and physical properties of the drugs
3. For targeting and retention of drug in blood circulation

1. For controlled release of drugs

1.1 To prolong the release rate

The release rate of drugs like withaferin and gliclazide from the niosomes was found slower as compared to other dosage forms.

1.2 In ophthalmic drug delivery

Experimental results of the water soluble antibiotic gentamicin sulphate showed a substantial change in the release rate. Besides this, the percent entrapment efficiency of gentamicin sulphate was altered when administered as niosomes. Also, as compared to normal drug solution, niosomes of drug show slow release.

Niosomal formulation containing timolol maleate (0.25%) prepared by chitosan coating exhibited more effect on intra ocular tension with fewer side effects as compared to the marketed formulation.

2. To improve the stability and physical properties of the drugs

2.1 To increase oral bioavailability

With the formulation of niosomes, the oral bioavailability of the acyclovir as well as griseofulvin was increased as compared to the drug alone. Similarly, the absorptivity of poorly absorbed peptide and ergot alkaloid can be increased by the administration in the bile duct of rats when administered as micellar solution together with the POE-24-cholesteryl ester.

2.2 For improvement of stability of peptide drugs

8- arginine, vasopressin, 9- glycinamide-ω

The *in- vitro* release of insulin from niosomes formulated by span 40 and span 60 in simulated intestinal fluid was lower than the niosomes formulated by span 20 and span 80.

Niosomes prepared by the span 60 has high resistance against proteolytic enzyme and exhibit good stability in storage temperature and in presence of sodium deoxycholate.

2.3 To promote transdermal delivery of drugs

Many drugs such as lidocaine, estradiol, cyclosporine etc are used for topical and transdermal drug delivery system by formulating them as Niosomes

The niosomes of natural compound, ammonium glycyrrhizinate were formulated for effective anti- inflammatory activity using new non- ionic surfactant, bola surfactant-span 80-cholesterol (2:3:1 ratio)

Experimental study showed that the bola niosomes were able to promote the intracellular uptake of ammonium glycerrhizinic acid

2.4 As a tool for improvement of stability of immunological products

Important tool for immunological selectivity, low toxicity and more stability of the incorporated active moiety

2.5 To improve anti- inflammatory activity

Niosomal formulation of Diclofenac sodium prepared with 70% cholesterol showed greater anti- inflammatory effect as compared to the free drug.

Similarly, nimesulide and flurbiprofen showed greater activity than the free drug.

3. For targeting and retention of drug in blood circulation

3.1 For increased uptake by A431 cells [a model cell line (epidermoid carcinoma) used in biomedical research]

Chitosan based vesicles incorporating transferrin and glucose as ligands have been reported. These vesicles bind CoA (co-A) to their surface. Chitosan containing vesicles are then taken up by A431 cells and the uptake was found to be enhanced by transferrin.

3.2 For liver targeting

Methotrexate was reported to be selectively taken up by liver cells after administration as a niosomal drug delivery system.

3.3 To improve the efficacy of drugs in cancer therapy

Niosomes can also be used as a suitable delivery system for the administration of drugs like 5-FU

Niosomes of doxorubicin prepared from C16 monoalkyl glycerol ether with or without cholesterol, exhibited an increased level of doxorubicin in tumor cells, serum and lungs, but not in liver and spleen

Niosomal preparation of methotrexate exhibited greater antitumor activity as compared to plain drug solution.

3.4 In treatment of localized psoriasis

In the treatment of localized psoriasis, niosomes of methotrexate taking chitosan as polymer have shown promising results.

3.5 In leishmaniasis

The leishmaniasis parasite mainly infects liver and spleen cells. The commonly used drugs, antimonials, may damage the body organ like heart, liver, kidney etc. The efficiency of sodium stilbogluconate has been found to be enhanced by incorporation in niosomes. The additive effect was observed for two doses given on successive days. Moreover, the distribution of antimony in mice showed the higher level of antimony in liver after its intravenous administration via niosomal drug formulation.

3.6 In diagnostic imaging

It has been studied that niosomes can also act as a carrier radiopharmaceuticals and showed site specificity for spleen and liver for their imaging studies using Tc labeled DTPA (diethylene triamine pentaacetic acid) containing Niosomes

Conjugated niosomal formulations of gadobenate with (N- palmitoyl- glucosamine, NPG), PEG 4400 and both PEG and NPG can be used to increased tumor targeting of a paramagnetic agent

3.7 Carrier for Haemoglobin

Niosomes play an important role as a carrier for haemoglobin. The niosomal haemoglobin suspension was found to give superimposable curve on free haemoglobin curve.

Usefulness of Niosomes in Cosmetics

- Niosomes of N- acetyl glucosamine are prepared due to its potential in the delivery of hydrophilic and hydrophobic drugs in topical form and improved penetration into the skin. Prepared formulations improved the extent of drug localized in the skin, as needed in **hyperpigmentation disorders.**[120]
- Elastic niosomes showed increased permeation through the skin which will be beneficial for **topical anti- ageing** application.
- Suitable for **skin moisturizing and tanning** products

- Niosomes were prepared as possible approach to improve the low skin penetration and bioavailability shown by conventional topical vehicle for **Minoxidil.**
- Niosomes with added solubilizers enhanced the permeation of ellagic acid (a potent **antioxidant phytochemical substance** which has limited use due to poor biopharmaceutical properties, low solubility and low permeability) into the skin with increased efficacy of ellagic acid.[121]

REVIEW OF LITERATURE- NIOSOMES

1. **Ismail A. Attia *et al.*[27]** formulated acyclovir niosomes in a trial to improve its poor and variable oral bioavailability by the conventional thin film hydration method using cholesterol, span 60 and dicetyl phosphate in the molar ratio 65:60:5 respectively. The formed unilamellar, spherical, shaped vesicles were in the size range 0.4 to 2.2 µm and exhibited significantly retarded release compared with free drug; and the *in- vitro* drug release profile was found to follow Higuchi's equation for free and niosomal drug. The *in- vivo* study revealed more the 2- fold increase in drug bioavailability in the niosomal formulation, which also showed significant increase in the mean residence time (MRT) of acyclovir reflecting sustained release characteristics. Thus it was concluded that the niosomal formulation could be a promising delivery system for acyclovir with improved oral bioavailability and prolonged drug release profiles.
2. **Vyas Jigar *et al.*[28]** formulated and evaluated topical niosomal gel of Erythromycin antibiotic to enhance skin penetration as well as to improve skin retention of the drug. Erythromycin was entrapped into niosomes by thin film hydration technique using span 20, span 60, span 80 and cholesterol; and various process parameters were optimized by partial factorial design. The optimized formulation was incorporated into carbopol gel and extensively characterized for percentage drug entrapment and *in- vitro* release performance. The stability of above formulation was studied at different temperatures. The study demonstrated prolongation of drug release, an increase in amount of drug retention into skin and improved permeation across the skin after encapsulation of Erythromycin into niosomal topical gel.
3. **Y. Prem Kumar *et al.*[29]** investigated the effectiveness of using Niosomes in a transdermal drug delivery system for Econazole, since topically applied Niosomes can increase the longer action on the skin (stratum corneum and epidermis), while reducing the systemic absorption of the drug. Econazole niosomes were prepared by thin film hydration technique by varying the cholesterol and span 80 ratios as 1:1, 1:2, 1:3 and 1:4. Each formulation was evaluated for drug release. The release showed required amount of drug release per day as well as extended for the required day is the optimized formulation. Hence, 1:4 ratio was concluded as best and was found to follow zero order release kinetics.

4. **R. Parthibarajan *et al.*[30]** designed Voriconazole niosomes by hand shaking and ether injection method using various ratios of span 80 and cholesterol and characterized the best formulation in terms of vesicle size distribution, entrapment efficiency and *in- vitro* release studies. Interaction of drug and the different ingredients in the niosomes were studied by FTIR spectra and found to be compatible. The entrapment efficiency and *in- vitro* drug release profile of voriconazole niosomes were determined by dialysis method. The evaluation results showed niosomes prepared by hand shaking method to be superior with entrapment efficiency of more than 84.53% and an entended release of 70.06% of drug up to 24 hours. It was concluded that *in- vitro* release of Voriconazole from niosomes was very slow when compared to the release from pure Voriconazole solution.
5. **Abdallah Marwa *et al.*[31]** studied the formulation of niosomes as carriers for delivery of diclofenac sodium using a series of sorbitan monoesters (Span 20, 40,60 and 80) and sorbitan trioleate (Span 85) and co- surfactants of polyoxyethylene fatty acid esters (Tween 20, 40, 60 and 80), with or without cholesterol and charged lipids like stearylamine or dicetylphosphate. The prepared niosomes were evaluated for entrapment efficiency and *in- vitro* release rate. Niosomes formed of span 60 gave the highest entrapment efficiency and showed the slowest release rate than those prepared from Span 20, 80 and 85. Niosomes prepared by ether- injection and reverse- phase evaporation method resulted in a marked increase in the entrapment efficiency compared to those prepared by hand-shaking method. It was also found that increasing the total lipid concentration resulted in an increase in the percentage entrapment efficiency, which also resulted while using co-surfactants like different types of Tween with Cholesterol in a molar ratio of 25:25:50. Hence, the incorporation of co- surfactants into niosomes resulted in a greater decrease in the release rate of diclofenac sodium from niosomal vesicles.
6. **Narayana Charyulu R *et al.*[32]** studied the influence of bio-enhancers on the release pattern of niosomes containing methotrexate by preparing sustained release formulations of niosomes of methotrexate alone and along with bio-enhancers by thin film hydration technique using span 60 as surfactant, cholesterol as membrane stabilizing agent, curcumin and piperine as bio-enhancers and dicetyl phosphate as charge inducing agent. All the formulations of niosomes were characterized on the basis of physical appearance and entrapment efficiency. The *in- vitro* release studies of optimized formulation of niosomes

were performed and compared with pure drug release. The entrapment efficiency of MTX with bioenhancers curcumin and piperine was found to be 40.30% and 69.1% respectively. *In- vitro*drug release of optimized formulation of MTX alone and with bioenhancers (F3) was found to be 98.89% and 60.97% at the end of 12 hours respectively. Results concluded that niosomes of MTX containing bioenhancers followed sustain release pattern.

7. **K. Sabarikumar *et al.*[33]** studied the bioavailability enhancement of Aceclofenac niosomes containing surfactants and cholesterol. The evaluation study included the effect of varying composition of non- ionic surfactant and cholesterol on the properties such as encapsulation efficiency, particle size, scanning electron microscopy and *in- vitro* drug release of Aceclofenac. The drug release was found to be modified and extended over a period of 72 hours in all formulations. The results of *in- vitro* drug release studies showed that formulation ANF-3 (Span 60 1:2:1) has better modified and extended release of drug (92.19%) for 72 hours. hence,ANF-3 emerged as the most satisfactory formulation in so far as its properties were concerned.
8. **Shete AS *et al.*[34]** developed and characterized PEGylated lipid coated niosomes of 5- fluoro uracil for parenteral drug delivery. The stealth niosomes were prepared by ether injection method using span 60 and the niosomal dispersion as bath sonicated for different period of time to reduce particle size at or above the phase transition temperature of surfactant. The performed niosomes were incubated with PEGylated lipid (5mol %) for 30 minutes. The un-entrapped drug was separated by centrifugation. It was observed that with increase in total molar concentration of surfactant and cholesterol, % entrapment efficiency increased. It was found that increase in sonication time reduces the particle size of the niosomes. PEGylated lipid coated niosomes were found to be larger than the conventional ones. The PEGylated lipid coated niosomes showed controlled *in- vitro* release of 5-FU for 6 hours. Stability study indicates that the PEGylated niosomes showed more drug retention stability than conventional niosomes.
9. **Prabagar Balakrishnan *et al.*[35]** performed formulation and *in – vitro* assessment of Minoxidil for enhanced skin delivery. Niosomes formed from Brij or Span with cholesterol molar ratios of 0,1,1.5 were prepared with varying drug amount 20-50 mg using thin film hydration method. The prepared systems were characterized for entrapment efficiency, particle size, zeta potential and stability. Skin permeation studies were performed using

static vertical diffusion Franz cells and hairless mouse skin treated with either niosomes, control Minoxidil solution or a leading topical Minoxidil commercial formulation (Minoxyl). Higher entrapment efficiency was obtained with the niosomes prepared from Span 60 and cholesterol at 1:1 molar ratio using 25 mg drug. Niosomal formulations have shown a fairly high retention of Minoxidil inside the vesicles (80%) at refrigerated temperature up to a period of 3 months. It was observed that both dialyzed and non-dialyzed niosomal formulations enhanced the percentage of dose accumulated in the skin compared to commercial and control formulations except dialyzed span 60 niosomes. The greatest skin accumulation was always obtained with non- dialyzed vesicular formulations. The results suggested that these niosomal formulations could constitute a promising approach for the topical delivery of Minoxidil in hair loss treatment.

10. **Sanket Dharashivkar *et al.*[36]** developed novel sustained release once a day niosomal formulation of Silver Sulphadiazine (SSD) in order to improve the patient compliance. Niosomes were prepared using different nonionic surfactants and cholesterol in the molar ratio of 1:1 by the ethanol injection method. Effect of different formulation variables like curing time, surfactant structure, HLB and molecular weight of a surfactant, on entrapment efficiency of SSD in niosomes were evaluated. Results indicated that the niosomes manufactured with span 60 gives highest entrapment and all niosomal formulations exhibited considerably retarded *in- vitro* release by a higuchi controlled mechanism. For span 60 niosomes the release was 98.14% over 28 hours. *In- vitro* antimicrobial study using *Pseudomonas aeruginosa* revealed that the niosomal formulation of SSD shows a better zone of inhibition (14mm) in comparison to conventional dosage form (12mm) even when used in half the concentration of conventional dosage form. This study showed that the niosomal formulation of SSD can be used as the promising sustained release approach for the topical delivery of SSD in burn treatment.
11. **Priya Hanu and Singh Harmanpreet[37]** formulated niosomes of Punicalagin, a hydrolysable tannin extracted from peels of *Punica granatum* in order to protect it from hydrolysis. The niosomes were formulated using varying amounts of span 60 and keeping the amount of cholesterol constant. The formulations were evaluated on the basis of morphology, entrapment efficiency, zeta potential and stability of the niosomes. As compared to other formulations F7 containing surfactant and cholesterol in ration 7:1 had

maximum entrapment efficiency of 65.93% and hence was selected as optimized formulated.

12. **Varaporn Buraphacheep Junyaprasert *et al.*[38]** investigated the influence of chemical penetration enhancers on the physicochemical properties of ellagic acid loaded niosomes. The ellagic acid niosomes were prepared by reverse phase evaporation method using Span 60, Tween 60 and cholesterol and Solulan C24 as a steric stabilizer. PEG was used as a solubilizer while DMSO or N-methyl 2- pyrrolidone (NMP) was used as a skin penetration enhancer. The formed spherical multilamellar vesicles were stable after 4 months storage at 4ºC. The DMSO niosomes showed the highest ellagic acid amount in epidermis; whereas the NMP niosomes had the highest ellagic acid amount in the acceptor medium. The results concluded that the DMSO niosomes were suitable for epidermis delivery of ellagic acid while the NMP niosomes can be used for dermis delivery of ellagic acid.
13. **Anchal Sankhyan and Pravin K Pawar[39]** prepared Metformin loaded non- ionic surfactant vesicles using thin film hydration technique and investigated for morphology, entrapment, *in- vitro* release, TEM and physical stability. Optimized formulation was further studied for the effect of surfactant concentration, DCP, surfactant: cholesterol ratio and volume of hydration. It was resulted that 100 molar concentration of cholesterol and surfactant, presence of DCP, equimolar ratio of span 60: cholesterol and 15ml of volume of hydration were found to be optimum for niosome preparation. The release studies data was subjected to release kinetics models. The present work concluded Metformin loaded niosomes to be effective in sustaining the drug release leading to decreased side effects and increased patient compliance.
14. **Shweta Patidar and Shelesh Jain[40]** investigated non ionic surfactant based vesicles containing Flupirtine Maleate as an Ocular drug delivery system to improve the low corneal permeability for effective management of trigeminal neuralgia. Niosomes formed immediately upon hydrating proniosomal gel which were developed with span 20, span 60, span 80, tween 20 and tween 80 with cholesterol, were characterized for morphology, entrapment efficiency, *in- vitro* drug release, drug release kinetics, stability, ocular irritation test and *in- vivo* eye wiping test for trigeminal neuralgia. The entrapment efficiency determined by centrifugation of freeze thawed vesicles followed the order span 80> span 60> span 20> tween 20> tween 80.the *in- vitro* release studies showed that there

was a prolonged release of drug which followed Higuchi model. Niosomes formed from span 80 and cholesterol s promising approach to prolong anti nociception activity and improve permeation rate as compared to pure drug.

15. **Tejaswi lella, M. Kishore Babu[41]** formulated Olanzapine niosomal suspensions by thin film hydration technique with a view to targeting the drug to the brain and produce a sustained release to increase the retention time of the drug in the brain. The higher values of entrapment efficiency were obtained with span 60, followed by span 20 and span 80. Drug entrapment efficiency was found to increase with increase in molar concentration of cholesterol. The highest percentages of drug release were obtained with niosomes prepared with span 60. The *in- vitro* diffusion studies followed first order kinetics and ascertained peppa's mechanism, governed by non- Fickian diffusion. High negative surface charge on niosomes by zeta analysis indicated high stability. The results concluded that the niosomal formulation F5 could be a better choice in the view of entrapment efficiency and drug release rate for the effective management of psychotic disorders.
16. **S. Dugal and A. Chaudhary[42]** investigated the potential of niosomes to aid in the slow sustained release of the topical antiseptic povidone- iodine (PVP- I) in order to overcome the major drawback of contact burns and other adverse reactions attributed to its sudden concentrated release from absorbent skin bandages. The niosomes were developed by the thin film hydration technique with stoichiometric proportion of antiseptic: cholesterol: sorbitan monooleate of 1:1:2; and was characterized for their particle size, zeta potential, drug entrapment efficiency, *in- vitro* agar cup tests and *in- vitro* drug release profile. The MIC of PVP- I for *Candida* was found to be 0.45% w/v of available iodine. The average size of loaded vesicles was 338.4 nm, with a drug entrapment efficiency of 80%. The encapsulated PVP- I showed good permeability, retention of the fungicidal activity and slow sustained effect lasted for almost 35 hours. The result demonstrated that niosomes can act as micro reservoirs to prolong the release of PVP- I and thus could help prevent contact burns associated with the immediate release of the antiseptic when used for topical application.
17. **Vijay S. Jatav *et al*.[43]** prepared rifampicin loaded niosomes by hand shaking method using surfactant and cholesterol in 50:60 percent mol fraction ratio. The percent of drug entrapped was noted to decrease progressively in the order span 85> Span 80> Span 60>

Span 40> Span 20; and the cumulative percent rifampicin released was maximum for Span 20 and minimum for Span 85 based niosomes. Thus it was concluded that handshaking method is a simple and efficient technique for designing functional niosomes for hydrophobic and amphiphilic drugs.

18. **Omar S. Salih *et al*.[44]** formulated a niosomal dosage form of rosuvastatin calcium with a view to improve its dissolution and permeability. The formulation was done by film hydration method using span 20, span 60 and span 80, cholesterol and lecithin in different ratios and evaluated in terms of assay by HPLC, particle size, morphology, *in- vitro* drug release and *ex- vivo* permeation study, SEM, TEM and FTIR. All formulas gave obvious morphology in the presence of cholesterol as a stabilizing agent and formula with span 60 had more entrapment efficiency than others with slower release after 7 hours *in- vitro* dissolution media. It was concluded that niosomes were promising dosage form to enhance dissolution and permeability of slightly soluble drugs prepared by film hydration method.
19. **Samyuktha Rani B and Vedha Hari B N[45]** prepared Orlistat niosomes from proniosome by reverse phase evaporation technique (slurry method) in rotary flash evaporator to improve its poor and variable oral bioavailability. The lipid mixture consisted of cholesterol, span 60 and beta cyclodextrin carrier in molar ratios 0.1:0.9:1 to 0.9:0.1:1 respectively. The niosomes were evaluated for particle size, entrapment efficiency, *in- vitro* drug release, release kinetics, FTIR, SEM, stability studies, conductivity and sedimentation rate, pH, density and viscosity. The niosomes had a mean size of 100nm with a smooth surface. Formulation OT9 was found to be the best with %EE of 44.09% and *in- vitro* release of 94.59% at the end of 12 hours. Release followed Hixson kinetics for diffusion and the niosomes had appropriate stability for 90 days at room temperature. Thus the niosomal formulations could be a promising delivery system for Orlistat with improved oral bioavailability, stability and for sustained drug release.
20. **Ye Jin *et al.*[46]** developed niosomal system to deliver *Gingko biloba* extract (GbE) with improved oral bioavailability and to replace the conventional GbE tablet. The GbE niosomes were prepared by film dispersion- homogenization method which was further freeze dried or spray dried to improve the stability of the niosomes. The characterization was done on the basis of morphology, particle size, zeta potential, entrapment efficiency, angle of repose, DSC, *in- vitro* release and *in- vivo* distribution study. The spray drying

method yielded niosomes with %EE of 77.5% with 50.1% for freeze dried niosomes. The niosomes showed a prolonged release up to 48 hours and the GbE niosomes were also detected in the rat brain tissue. Hence it was concluded that niosomes are a promising oral system for delivery of GbE to the brain.

REVIEW OF LITERATURE- KETOCONAZOLE

1. **R. Shireesh kiran[89] et al**. had investigated the ketoconazole nail lacquer as ungual drug delivery system for the treatment of onychomycosis. Topical therapy of nail diseases is limited by the poor permeability of nail plate. they had used few ungual enhancers like thioglycollic acid (TA) and urea hydrogen peroxide and investigated their effect on the nail permeation of ketoconazole drug. *In-vitro* drug permeation studies were carried out across human nail plates using Franz diffusion cells. They stated that significantly higher permeation was achieved in the presence of thioglycollic acid as enhancer.

2. **Junxiu Che et al.** had investigated to develop a ternary skin targeting system for ketoconazole using a combined strategy of micro emulsion and cyclodextrin (beta-hydroxyl cyclodextrin) i.e KET-CD-ME. The KET-CD-ME was formulated using Labrafil M 1944 CS as oil phase, Solutol HS 15 as surfactant, Transcutol P as cosurfactant, and HP-β-CD solution as aqueous phase.*in-vitro* sensitivity against candida parapsilosis test indicated that KET-CD-ME enhanced KET anti- fungal activity mainly owing to the solubilisation of HP-β-CD on KET in the ternary system.

3. **M Najmuddin et al. [91]**formulated the solid dispersion incorporated gel of ketoconazole. The solubility of ketoconazole was increased by complexation with β cyclodextrin which was prepared by solvent evaporation technique and then incorporated into gels. Ketoconazole gel formulations were made with different polymers like carbopol 940, HPMC, Methyl cellulose and sodium carboxy methyl cellulose containing various permeation enhancers like sodium lauryl sulphate, dimethyl sulfoxide in different proportions. The formulated gels were evaluated for various physiochemical parameters

and showed good spreadability, extrudability. The carbopol 940 with 15% of dimethyl sulfoxide showed better drug release at the end of 6 hrs.

4. **P.M. Satturwar et al.**[112] had prepared Ketoconazole niosomes by ether injection technique using surfactant (Tween 40 or 80), cholesterol and drug in five different ratios by weight. They were characterized for size, shape, entrapment efficiency and *in-vitro* drug release (by exhaustive dialysis). The formulations were also tested for *in-vitro* (cup plate method) and *in-vivo* anti – fungal activity (in rabbits) and compared with free ketoconazole. The results indicate that niosomes have potential to reduce the therapeutic dose of ketoconazole by improving its performance.
5. **Rakesh P.Patel et al.**[93] had formulated ketoconazole liposomes by thin film hydration technique using soya lechithin, cholesterol and drug in different ratios. The prepared liposomes were characterized for size, shape, entrapment efficiency, *in-vitro* drug release (by Franz diffusion cell) and physical stability. The studies demonstrated successful preparation of ketoconazole liposomes and effect of soya lecithin: cholesterol weight ratio on entrapment efficiency and on drug release.
6. **Dattatreya B Udgirkar et al**.[95] had formulated buccoadhesive tablets of ketoconazole by using HPMC K4M and Carbopol 934 P as bio adhesive polymers and Ethylcellulose (EC) as baking material. The solubility of ketoconazole was increased by inclusion complex with β-cyclodextrin and then delivery via buccal mucosa. The bucco adhesive tablets for the delivery of ketoconazole were prepared by 3^2 factorial design; Direct compression of HPMC K4M and Carbopol 934P. the results of various studies revealed that there was increase in drug release rate from the tablets in solution as well as permeated through sheep buccal mucosa and showed better potential for buccal administration.
7. **Rahul R. Patel et al.**[96] had developed micro emulsion based gel (MBG) formulation of ketoconazole for enhancing its solubility and permeability. **MBG** was prepared with Capmul MCM NF as oil, Acrysol K150 as surfactant, propylene glycol as co-surfactant, carbopol as gelling agent. They had optimized the system by ternary phase diagram. They had analysed the formulations for Drug content, % transmittance, visual assessment, particle size and zeta potential analysis. *In-vitro* release of the formulations showed better release than the marketed formulation. Hence, it has significant increase in solubility of ketoconazole.

8. **M. ABD Elgadir et al.[98]** had investigated the relationship between water number value of selected ointments and release of ketoconazole as a model drug. They had used the ointment bases like soft paraffin, wool fat, cetostearyl alcohol, and soft paraffin. They had stated in their results that the addition of a fatty alcohol as cetostearyl alcohol to soft paraffin has increased the water number from 11.5 to 57.5 ml.
9. **Mirela Adriana Mitu et al.[97]** had investigated the influence of the receptor media on the *in-vitro* diffusion kinetics of Ketoconazole topical pharmaceutical formulations using a vertical diffusion cell system and two types of receptor media which differs by the solubilizing capacity for the active pharmaceutical ingredient. The experimental results indicated the impact of both composition of the receptor media and nature of the pharmaceutical dosage form on the dissolution profiles.
10. **M. Skiba et al.[113]** had investigated the stability assessment of ketoconazole in aqueous formulations. The stability of ketoconazole in aqueous media was assessed as a function of pH, antioxidant and ketoconazole concentration.it was found that ketoconazole was least stable at pH 1. The viscosity of the formulation was found to be more stable at high pH because carbopol is stable at basic pH and protected ketoconazole. It appears that the amount of ketoconazole in the formulation has a low influence on the degradation stability of ketoconazole.
11. **Nagoba S.N. et al.[99]** formulated ketoconazole as a tablet lozenge to provide slow release medicament for the treatment of oral thrush in pediatric patients. The lozenges were prepared by heating and congealing method in a candy base. the prepared formulations were subjected to various physiochemical parameters like hardness, content uniformity, friability, weight variation, stability studies etc. In all the tests performed, the lozenges show better results and could be an attractive alternative formulation in the treatment of oral thrush in pediatric patients.
12. **Rolando E. Saenz et al. [86]** had compared the efficacy of anti-fungal drug ketoconazole (600 mg/day for 28 days) with a recommended regimen of intramuscular pentostam in a randomized study of the treatment of Panamanian cutaneous leishmaiasis due to leishmania braziliensis panamensis. Ketoconazole clinically cured 16 of 21 patients. Both keoconazole and pentostam were more effective than placebo against L. braziliensis panamensis cutaneous leishmaniasis.

LITERATURE REVIEW ON NIOSOMAL GEL

1. **Loveleen Preet Kaur et al.**[115] had reviewed the topical gel formulations in the novel drug delivery system. Gel formulation provides better application property and stability in comparison to cream and ointment. Topical application of drugs offers potential advantages of delivering the drug directly to the site of action and acting for an extended period of time. They had presented various classification of gels, method preparation, various evaluation parameters etc. Gels are evaluated by following parameters such as pH, homogeneity, grittiness drug content, viscosity, spreadability, extrudability, skin irritation studies, in-vitro release, in Stability
2. **P.S. Salve**[117] had developed a topical drug delivery system using niosomal gel. Niosomes of terbinafine hydrochloride was prepared by thin film hydration method in size range of 0.24 to 9.4 µm. zeta potential of niosomes containing span 60 (1:1) were more stable than other formulations. Niosomes were incorporated in 1.5 % w/v carbopol gel at pH 6.8 to 7.0. In *in-vitro* anti-fungal against candida albicans, vesicular systems are more effective than conventional gel. In *ex-vivo* percutaneous permeation studies, niosomal formulations have shown superior skin penetration and drug deposition. The formulation containing tween 80 has shown higher drug deposition in rat skin as compared to other formulations.
3. **V.Sathyavathi et al.**[118] developed niosomal *in-situ* gel for glaucoma treatment. The problem of poor bioavaiability of ocular drugs due to tear production, non-productive absorption, transient residence time can be minimized by the use of niosomal vesicular system. niosomes were formulated by using different ratios of span series and cholesterol. *In-situ* gelling of niosomal drops was formulated by using HPMC K 15 M and carbopol 940 to maintain the drug localization for extended period of time. All the gel formulations exhibit pseudo plastic rheological behavior and slow drug release pattern. Anti-glaucoma activity of the prepared gel formulations showed more significant and sustained effect in reducing intra ocular than marketed and niosomal drops.
4. **T.Coviello et al.**[119] had prepared gels with xanthan and Locust bean gum and loaded with nonionic surfactant vesicles., the vesicles composed by Tween 80 and cholesterol or by tween 85 and span 20 were loaded with monosodium glycyrrhizinate for release experiments. Size and zeta potential of the vesicles were evaluated and the new systems

were characterized by rheological and dynamo-mechanical measurements. For an appropriate comparison, a carbopol gel and commercial gel were also evaluated. *In-vitro* experiments showed that the polysaccharide network protects the integrity of the vesicles and leads their slow release without disruption of the aggregated structures. Further more, being the vesicles composed of molecules possessing enhancing properties, the permeation of the loaded dugs topically delivered can be improved. thus, the new system combine the advantages of matrices for a modified release (polymeric component) and those of an easier permeability

5. **Jigar Vyas et al.[116]** had entrapped Benzoyl peroxide into niosomes by thin film hydration technique and various process parameters were optimized by partial factorial design. The optimized niosomal gel formulation was incorporated into HPMC K15 and extensively characterized for percentage drug entrapment and *in-vitro* release performance. Their study had demonstrated prolongation of drug release, increased drug retention into skin, and improved permeation across the skin after encapsulation of benzoyl peroxide into niosomal gel.
6. **P.K.Lakshmi et al.[114]** had prepared methotrexate niosomes by lipid layer hydration method. The characterized niosomes were incorporated into chitosan gels. The gels were tested for irritation and skin sensitivity by human repeated insult patch test. The formulations were assessed for efficacy by double blind placebo controlled study in 10 psoriasis patients for each formulation. The results suggest that niosomal methotrexate gel is more efficacious than placebo and marketed methotrexate gel.

REVIEW OF LITERATURE ON LEISHMANIASIS

1. **Ramzi A.Mothana et al.[80]** evaluated the anti-plasmodial, antileishmanial and antitrypanosomal activity of twenty-five medicinal plants distributed in Saudi Arabia and Yemen. The plants were extracted with methanol and screened *in-vitro* against erythroytic schizonts of plasmodium falciparum, intracellular amastigotes of Leishmania infantum and trypanosomeruzi and free trypomastigotes of T.brucei. Interesting activity against L.infantum was obtained with Verbascum bottae and Solanum glabratum(IC_{50} 8.1 µg/ml,SI 3.4). these results partly support the traditional use of some of the selected medicinal plants and warrant further investigations into the putative active constituents.

2. **S.Lala et al.**[81] investigated the *in-vitro* anti-leishmanial activity of 14-deoxy-11-oxoandrographolide a derivative of andrographolide, isolated from the Indian medicinal plant Andrographis paniculata in different vesicular delivery modes on hamster model of Leishmaniasis. The drug in various delivery modes, particularly in liposomal and niosomal forms showed apparently no immediate toxicity. Hence, these forms of 14-deoxy-11-oxoandrographolide might have clinical application to combat visceral Leishmaniasis.
3. **Payam Khazaeli et al.**[82] had prepared and evaluated the effect of itraconazole niosome on the in-vitro susceptibility of Leishmania tropica as compared to itraconazole alone or tartar emetic. The overall growth rate of promastigotes treated with various concentrations of itraconazole niosomes was significantly lower than itraconazole alone. Their findings indicated that niosomes could be developed as novel drug delivery for itraconazole in the in-vitro model.
4. **Heibatullah Kalantari et al.**[87] had investigated the effect of topical nanoliposomes of anti-leishmanial drug Paromomycin on rats liver and kidney. They had evaluated the probable nephrotoxicity and hepatotoxicity of topically administered PMS liposomes. their report states that the application of nanoliposomal paromomycin sulfate formulations for topical treatment of the cutaneous leishmaniasis does not create serious side effects in the short term use.
5. **Umakant Sharma et al.**[85] had investigated the antiparasitic activity of plumericin & isoplumericin isolated from Plumeria bicolor against Leishmania donovani. The plumeria bicolor extract showed activity with the IC_{50} of 21±2.2 and 14±1.6 µg/ml against promastigote and amastigote forms, respectively. Hence, their results indicated plumericin has a promising anti-leishmanial activity.
6. **Luciana S.Ferreira et al.**[84] had investigated the *in-vitro* skin permeation and retention of paromomycin from liposomes for topical treatment of the cutaneous leishmaniasis. Liposomal formulation usually provide sustained and enhanced drug levels of paromomycin. Controlled topical delivery, across stripped skin was observed for PA entrapped in liposomes.
7. **Rolando E. Saenz et al**[86] had compared the efficacy of anti-fungal drug ketoconazole (600 mg/day for 28 days) with a recommended regimen of intramuscular pentostam in a randomized study of the treatment of Panamanian cutaneous leishmaiasis due to leishmania

braziliensis panamensis. Ketoconazole clinically cured 16 of 21 patients. Both keoconazole and pentostam were more effective than placebo against L. braziliensis panamensis cutaneous leishmaniasis.

8. **Elena pinelli et al.**[111] had used the canine macrophage cell line (030-D) that can readily be infected with Leishmania infantum. Their objective is to further characterize the effector mechanisms involved in killing of Leishmania parasites in dogs. They had observed that activation of 030-D cells resulted in enhanced nitric oxide (NO) productionby these cells.hence, they proposed the infection of the 030-D cell line as a good *in-vitro* model to further investigate parasite-host cell interactions in dogs.

3. AIM AND PLAN OF WORK

AIM OF WORK

The aim of the present study is

- To formulate Ketoconazole niosomal gel drug delivery system using various non -ionic surfactants and cholesterol to enhance its therapeutic efficacy by controlled release.
- To provide an efficient dosage form by targeting it to the macrophages for the treatment of Cutaneous Leishmaniasis.

PLAN OF WORK

The present work was designed and planned as follows:

- Drug excipients compatibility studies- FT- IR study
- Determination of λ_{max} of Ketoconazole in phosphate buffered saline pH 7.4
- Calibration curve of the drug in phosphate buffered saline pH 7.4
- Preformulation studies and optimization of process related variables using niosomes prepared in different molar ratios of non- ionic surfactant (Span 40) and constant molar ratio of cholesterol (25 µmol) by thin film hydration technique.
  - Effect of hydration time
  - Effect of capacity and rotational speed of evaporator flask
  - Effect of Sonication time
  - Effect of osmotic shock
- Formulation of Ketoconazole niosomes using optimized process parameters using various molar ratios of different non- ionic surfactants keeping cholesterol constant at 25 µmol.
- Evaluation of Ketoconazole niosomes
  - Drug content
  - Entrapment efficiency by centrifugation method
  - *In- vitro* drug release study of niosomal formulations and Ketoconazole drug solution in phosphate buffered saline pH 7.4 using dialysis membrane
  - *In- vitro* release kinetics
- Selection of best ratio and preparation of Ketoconazole niosomal gel using various non- ionic surfactants (Span 20, Span 40 and Span 60).
- Determination of particle size distribution using Malvern zeta analyzer.
- Morphological studies using Optical microscopy and Scanning electron microscopy(SEM).

- Formulation of Ketoconazole Niosomal gel using the optimized ratio of surfactants.
- Evaluation of Ketoconazole Niosomal gel.
    - Physical appearance
    - pH of the gel
    - Rheology – Brookefield Viscometer
    - Drug content estimation
    - Entrapment Efficiency
    - *In-vitro* Drug Diffusion study.
- Stability study of the optimized niosomal gel formulations.

4. RATIONALE OF STUDY

RATIONALE OF THE STUDY[6, 76, 81, 82, 83]

Leishmaniasis is one of the world's most neglected diseases largely affecting the poorest of the poor, mainly in developing countries. Over 350 million people are considered at risk of contracting leishmaniasis and approximately 2 million new cases occur yearly.[83] Although Cutaneous Leishmaniasis (CL) is a self- healing disease, it can result in disfiguring scar and long-lasting stigmas, which may destroy underlying structures like nose, ear or the exposed sites of skin which causes the psychological suffering of patients.[82]

Although Pentavalent antimonials (SbV) are still considered the first line of treatment for all forms of Leishmaniasis, there are some reports of drug resistance and unresponsiveness to the treatment with these drugs. While, the second line of drugs such as pentamidine and amphotericin B have low efficacy with side effects. Therefore it is desirable that new drugs as well as new delivery systems developed to enhance the efficacy and to reduce the toxicity.[81]

Anti-fungal agents including azole derivatives (fluconazole, econazole, miconazole, ketoconazole and itraconazole) have had clinically acceptable leishmanicidal activities.

RATIONALE FOR SELECTION OF DRUG [76, 122]

Ketoconazole is a synthetic imidazole anti-fungal drug used primarily to treat fungal infections.[122] It has shown activity against Leishmaniasis by interfering with the sterol biosynthesis present in the leishmanial membranes. Like fungi, Leishmania synthesize 24-substituted sterols, such as ergosterol (mammals have cholesterol). Ketoconazole inhibit a key enzyme of this pathway 14 α demethylase. Ketoconazole has given equivocal results in trials against both CL and VL.[76]

RATIONALE FOR SELECTION OF DOSAGE FORM [6, 24]

The purpose of this research work was to formulate and optimize the niosomal gel drug delivery system containing Ketoconazole to enhance its therapeutic efficacy by controlled release and by targeting it to the reticulo endothelial system (RES). Vesicular drug delivery system plays a major role in modeling biological membranes and in the transport and targeting of active agents.

Passive capture of colloidal carriers by macrophage offers therapeutic opportunities for the delivery of anti-infectives for disease conditions that involve macrophage cells of the reticuloendothelial system (RES) e.g., leishmaniasis.[6]

Niosomes have been reported to offer the following advantages

- Targeted drug delivery
- Protection of drug
- Increased bioavailability
- Sustained release
- Enhanced cellular uptake[6]

The above aspects led to the formulation of Ketoconazole niosomal gel drug delivery system which establishes and maintains the drug concentration at the target site for a prolonged period of time.

NEGLECTED TROPICAL DISEASES[109]

Figure 4 Cutaneous Leishmaniasis. "Jericho buttons"

Neglected tropical diseases (NTDs) are a diverse group of communicable diseases that prevail in tropical and subtropical conditions in 149 countries and affect more than one billion people, costing developing economies billions of dollars every year. They mainly affect populations living in poverty, without adequate sanitation and in close contact with infectious vectors and domestic animals and livestock.[109]

A subset of life-threatening NTDs includes Kala azar (visceral leishmaniasis), sleeping sickness (African trypanosomiasis) and Chagas disease (American trypanosomiasis), Dengue, Chikungunya, river blindness (Onchocerciasis) which all may lead to fatal complications but are restricted to limited geographical areas and specific groups. Most current chemotherapeutics against these diseases are toxic, marginally effective and must be given by injection and become compromised by the development of drug-resistance. Despite the large number of people at risk and the substantial burden of disease, with few exceptions, no major interventions have been developed for generations. [80]

LEISHMANIASIS

Leishmaniasis has been known for many hundreds of years, with one of the first clinical descriptions made in 1756 by Alexander Russell and called Aleppo boil. Many names correspond to this group of diseases: kala-azar, dum-dum fever, white leprosy, espundia, pian bois, and so on. Leishmaniases are parasitic diseases spread by the bite of the infected female phlebotomine sand fly (Fig.5.).[78]

Figure 5. Phlebotomus Sandfly female

ETIOLOGY

Leishmaniases are caused by infection of various species of Leishmania, a protozoan parasite of the family trypanosomatidae (order kinetoplastida).[108]The trypanosomes are characterized by having a single long whip-like projection known as a flagellum. Members of this group are parasitic, mostly on insects, but several genera have life cycles that involve alternative hosts including plants and vertebrates. The latter group include the species that can cause severe diseases in humans including Leishmaniasis (*Leishmania* spp), African sleeping sickness and Chagas disease (*Trypanosoma* spp.),and others.[79]

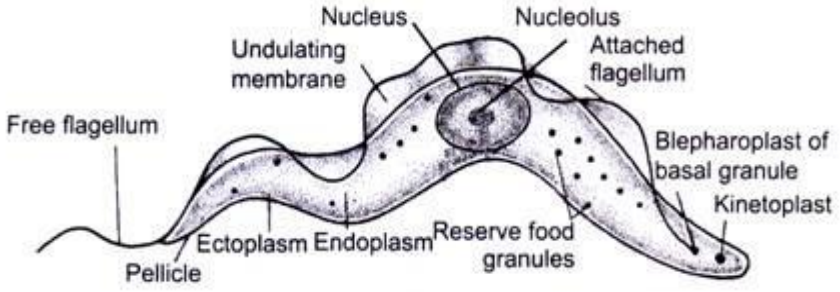


Fig.6.A Trypanosoma

Human visceral leishmaniasis is primarily caused by Leishmania donovani (which includes L. archibaldi) and L. infantum/ L. chagasi. L. donovani is anthroponotic it is mainly transmitted between people, who act as the reservoir hosts. L. infantum is zoonotic. At one time, two different names were used for this organism - L. infantum in the "Old World" (Eastern Hemisphere) and L. chagasi in the "New World" (Western Hemisphere) – and these two organisms were thought to be different species. As a result of genetic studies, they have been reclassified into one species, L. infantum. However, some authors argue that L. chagasi should be a subspecies of L. infantum, and the name L. chagasi is still used frequently in South America. Other organisms can occasionally cause visceral leishmaniasis with L. tropica and L. amazonensis, which usually cause cutaneous leishmaniasis, and a newly described species in Thailand, have been linked to some cases.

Most Leishmania species cause cutaneous leishmaniasis in people. In the New World, these organisms include the members of the L. braziliensis complex (L. braziliensis, L. panamensis/ L. guyanensis, L. shawi and L. peruviana) and the L. mexicana complex (L. mexicana, L. amazonensis, L. venezuelensis), as well as L. lainsoni, L. naiffi and L. lindenbergi. Old World

species that cause cutaneous leishmaniasis include L. tropica, L. major and L. aethiopica, which are all members of the L. tropica complex. In addition some strains of L. infantum can cause cutaneous leishmaniasis without affecting the internal organs. With the exception of the anthroponotic species L. tropica, all of these organisms are zoonotic. The type of skin lesions, efficacy of treatment, speed of healing and other factors vary with the species. Most Old World and New World species only cause lesions on the skin, but the New World organisms L. braziliensis and L. panamensis/L. guyanensis may cause either cutaneous or mucocutaneous leishmaniasis.[108]

Classification[79]

There are four major forms of human leishmaniasis. The particular presentation of the disease reflects a complex interplay between the individual infecting species and the host's immune response.

• **Cutaneous leishmaniasis** (CL - Baghdad ulcer, Delhi boil, *Bouton d' Orient*) - is the most common form of the disease and produces large numbers of skin lesions that self-heal within a few months but can leave many unsightly and sometimes disabling scars.

• **Diffuse cutaneous leishmaniasis** (DCL) – produces disseminated and chronic skin lesions that do not heal spontaneously and tend to relapse after treatment.

• **Mucocutaneous leishmaniasis** (MCL - espundia) - begins with skin ulcers and progresses to lesions which cause massive tissue destruction of the mouth, nose and throat cavities and severe disfigurement.

• **Visceral leishmaniasis** (VL - *kala azar*, black fever) – is the most serious form of the disease and causes death in almost 100% of the cases if left untreated. Symptoms include significant swelling of the spleen and liver, irregular fever episodes, substantial weight loss, decreased appetite, anemia, abdominal distension with splenomegaly and hepatomegaly. Progress of the disease is extremely variable. The usual duration is 12-16 weeks, but individual cases can last from one to more than 20 weeks. People with successfully treated infections continue to carry the parasite, and the disease may recur if they become immune-supressed.

Sometimes a secondary form of the disease called **post kala-azar dermal leishmaniasis** sets in a few months to several years after recovery from VL. The disease starts as small skin lesions on the face which gradually enlarge and spread over the body. The lesions may eventually form disfiguring swollen structures that may cause blindness if they reach the eyes. This condition is distinct from the milder cutaneous leishmaniasis. In India, PKDL is seen in 1-3% of successfully treated cases of visceral leishmaniasis.

Cutaneous leishmaniasis:

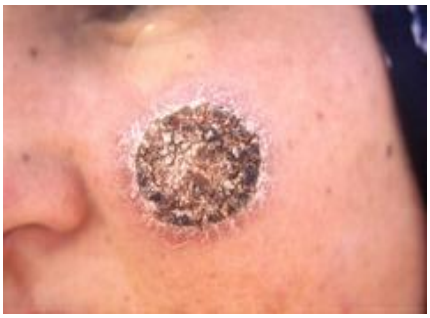


Figure 7. A Delhi boil

It is also known as **oriental sore**, **tropical sore**, **Delhi boil, Baghdad ulcer, chiclero ulcer**, or **chiclero's ulcer** is the most common form of leishmaniasis affecting humans. It typically presents as a papule that enlarges over weeks to months to form a shallow ulcer with raised red margins and that is thought (without definitive data) to ultimately self-heal with scarring in months to years.

Many lesions remain localized, but in some cases, the parasites may spread via the lymphatics and produce secondary lesions on the skin or occasionally the mucosa of other parts of the body. Regional lymphadenopathy sometimes occurs. Cutaneous leishmaniasis is usually painless unless the lesions become secondarily infected and except in the ear, the ulcers tend to remain confined to the skin and do not affect the subcutaneous tissues. Most skin lesions heal spontaneously however, the speed of healing varies with the species of *Leishmania*. In some cases, it may take several months to a year or longer. Some forms leave permanent scars. HIV-infected individuals can have unusually severe cases, and the disease is more difficult to cure. Steroid treatment or other forms of immunosuppression can also result in unusually severe disease.

In cutaneous disease due to Leishmania braziliensis species, the ulcers may weep and the disease may metastasize to the proximal skin or rarely to the oronasal mucosa (muosal leishmaniasis).

Disseminated leishmaniasis[108] is a rare form of cutaneous disease. It is seen especially with *L. amazonensis* in the Western Hemisphere, although other organisms can also be involved. It also occurs in the Eastern Hemisphere, often in people who have concurrent HIV infections. In diffuse cutaneous leishmaniasis, the nodules do not ulcerate but they spread widely on the skin. They may cause damage to deep tissues, and can persist indefinitely. The diffuse form can be incurable in some cases.

Leishmaniasis recidivans[108] (lupoid leishmaniasis), another rare form, is characterized by the development of new lesions around the edges of a healed skin lesion. It is most often caused by *L tropica* or *L braziliensis* and it does not heal without treatment.

Although cutaneous leishmaniasis results in morbidity rather than in mortality, such patients are generally treated because of the local morbidity and the possibility of metastasis.

VARIOUS STAGES OF LIFE CYCLE OF LEISHMANIASIS

Leishmania spp. are digenetic or heteroxenous parasites, whose life cycle involves two hosts, a vertebrate and an invertebrate, the sandfly. Hemoflagellates may have several morphological

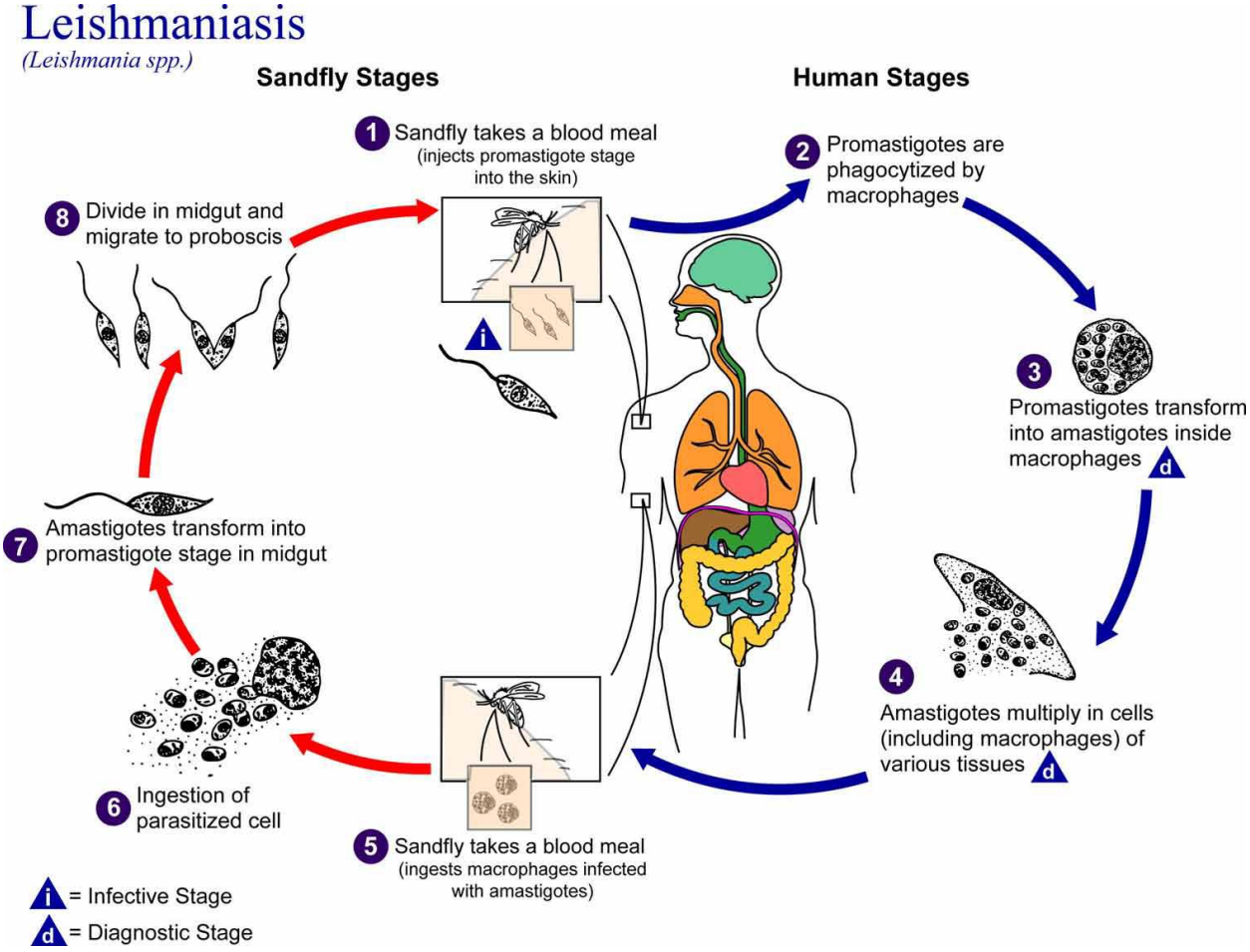


Figure 8 stages of life cycle - Leishmania spp

stages that differ from one another in the placement of the kinetoplast relative to the nucleus, as well as the location and origin of the flagellum. *Leishmania* exist in two basic body forms, the amastigote, the intracellular form in the vertebrate host and the promastigote, the extracellular form in the sandfly (*Phleobotomus* spp. and *Lutzomyia* spp) vector. Amastigotes are taken up from the blood of an infected host when the female sandfly bites and in the sand-fly gut they develop into promastigotes where they multiply by binary fission, pro-mastigotes move anteriorly into the proboscis and are introduced into the vertebrate host when the sand-fly bites again. The

promastigotes injected by the sand-fly during feeding are phagocytized and develop into intracellular amastigotes.

The amastigote, literally "without a flagellum," is the intracellular, non-motile form in the vertebrate host and it divides by longitudinal binary fission at 37°C. Intracellular amastigotes are 3-6 um in length and 1.5-3.0 um in width. The amastigote is also called the Leishman-Donovan (LD) body. The amastigote is not really devoid of a flagellum, it is simply that the flagellum does not protrude beyond the body surface and by light microscopy cannot be seen.

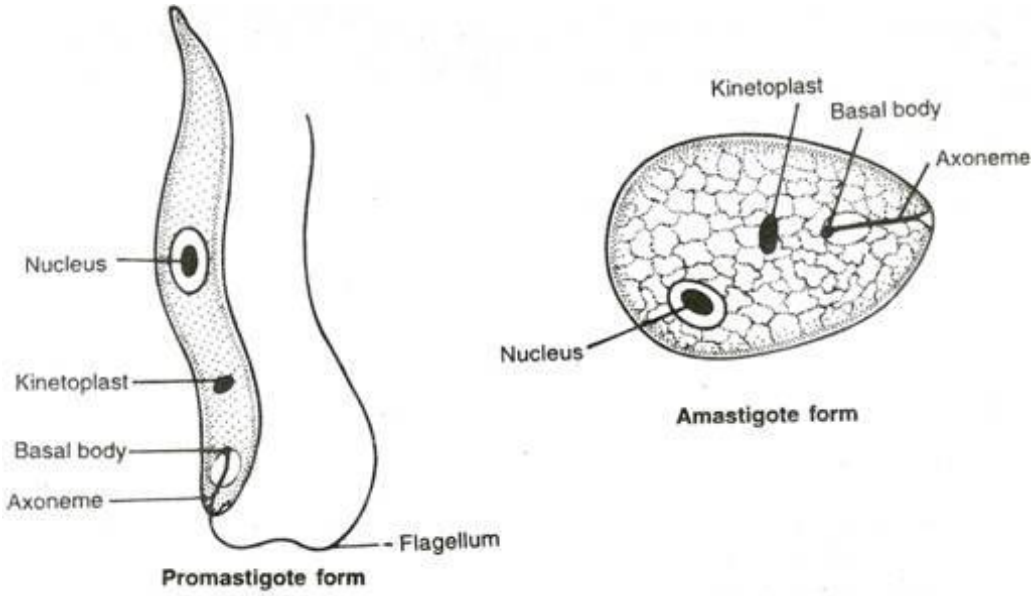


Figure 9. Morphological forms of Leishmania

The promastigote, literally the body form with "an anterior flagellum" is 15-30 um in body length and 5mm in width; it is extracellular, motile, and grows and divides by longitudinal binary fission at 27°C in the sand-fly. Promastigotes can be grown *in- vitro* at 25°C temperature on NNN medium, which has a solid phase of blood agar and a liquid phase containing a physiologic salt solution. Liquid media that support promastigote growth are also available. Amastigotes usually are grown inside tissue culture cells and can also be grown extra-cellularly at 37°C under special conditions.[108]

Note: Most *Leishmania* species are maintained by an animal-to-animal transmission cycle and humans are considered an accidental host. However, anthroponotic transmission without animal reservoirs is also reported in some *Leishmania* species.

The transformation from amastigote to promastigote in the gut of the sandfly is triggered by a change in conditions such as temperature and pH. The main target of the parasite in mammalian

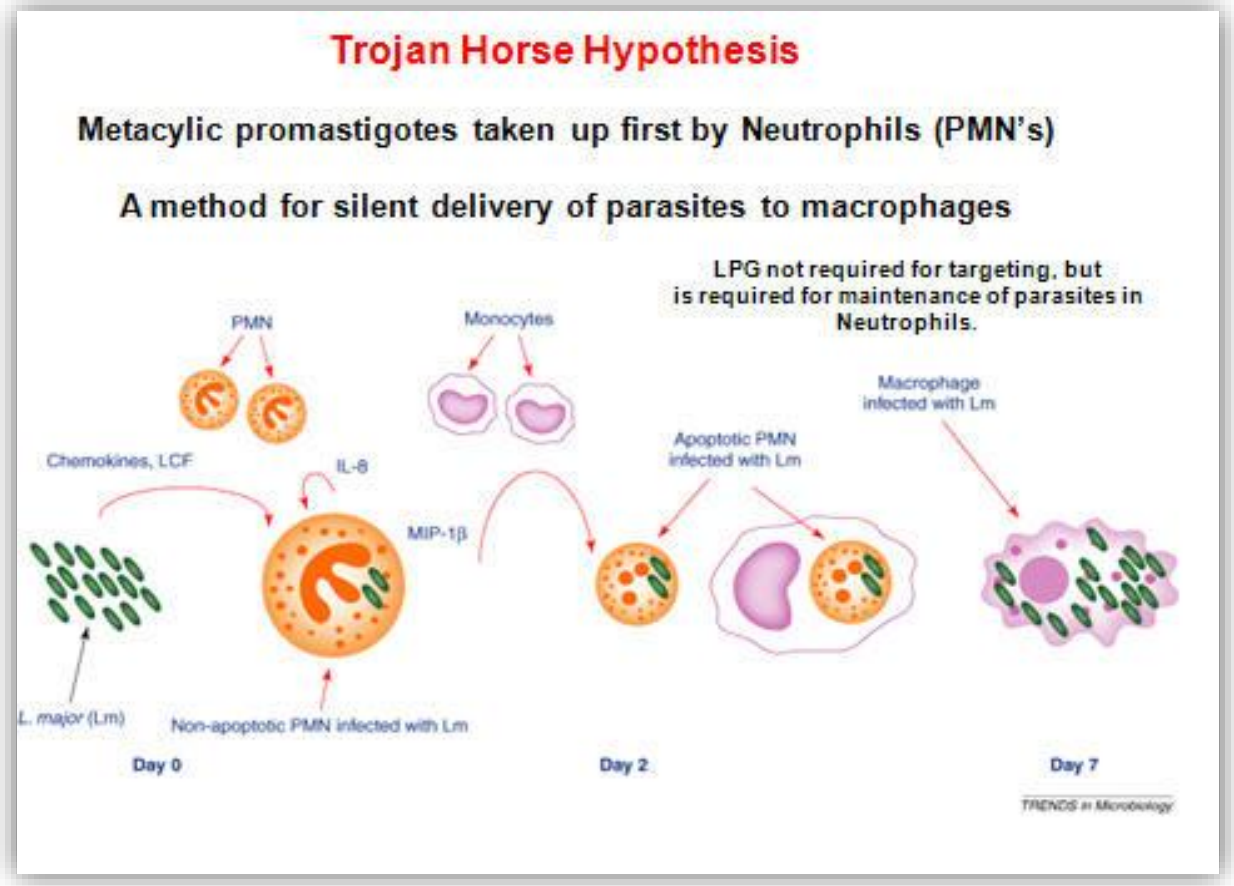


Figure 10 Trojan Horse Hypothesis.[110]

hosts is macrophages, and infections are known to occur in three ways (1) direct infection, (2) phagocytosis of infected neutrophils by macrophages (the Trojan horse model) and 3) silent infection by parasites released from apoptotic neutrophils. Neutrophils which are recruited to sites of tissue damage caused by sand fly bites in the initial phase, are considered to ingest the majority of parasites and play a central role in the establishment of the *Leishmania* infection .[103]

Communicability[108]

Leishmaniasis is usually vector-borne, but person-to-person transmission including vertical (congenital) transmission, venereal transmission, and transmission by blood transfusion has been reported. Newborns can be infected whether or not the mother was symptomatic. Humans infected with some species of *Leishmania* can infect sandflies.

Transmission[108]

Leishmania spp are usually transmitted indirectly between hosts by sandflies of the genera *Phlebotomus* and *Lutzomyia*, which are biological vectors. Each species of *Leishmania* is adapted to transmission in certain species of sandflies. Only the females feed on blood. Sandfly activity occurs when it is humid, and there is no wind or rain. These insects are usually most active at dawn, dusk and during the night, but they will bite if they are disturbed in their hiding places (animal burrows, holes in trees, caves, houses and other relatively cool, humid locations) during the day. They are attracted to light and may enter buildings at night.

Other arthropods including ticks (*Dermacentor variabilis* and *Rhipicephalus sanguineus*) and canine fleas may also act as mechanical vectors. Where sandflies transmit *Leishmania* spp., ticks and fleas are probably unimportant in the epidemiology of the disease, however they might be involved in rare cases of dog-to-dog transmission in other locations.

These parasites have also been transmitted via blood transfusions in people and dogs and by transplacental transmission in dogs, mice and humans. In canine leishmaniasis caused by *L. infantum*, the parasites can sometimes be found in saliva, urine, semen and conjunctival secretions, as well as in blood.

Diagnostic tests[108]

Cutaneous leishmaniasis can be diagnosed by direct observation of the parasites in skin scrapings, impression smears or skin biopsies stained with Giemsa, Leishman's, Wright's or other stains. Amastigotes are easiest to find in recent or active lesions. Polymerase chain reaction assays (PCR) are often used for diagnosis in areas where they are available. *Leishmania* spp can also be cultured. However, each species will grow only in certain media and some species can be difficult to isolate. Novy-MacNeil-Nicole (NMN) medium, brain–heart infusion (BHI) medium, Evan's modified Tobie's medium (EMTM), Grace's medium and Schneider's *Drosophila* medium might be used initially. Animal inoculation into hamsters may also be valuable, especially with contaminated material. Diagnosing leishmaniasis by *in vitro* culture requires 5 to 30 days, while animal inoculation can take weeks or months. The species, subspecies and/or strain can be identified by PCR, DNA hybridization, kinetoplast DNA restriction endonuclease analysis, isoenzyme analysis or immunological techniques that use monoclonal antibodies. A delayed hypersensitivity test, the leishmanin skin test (Montenegro skin test), is useful in the diagnosis of cutaneous and

mucocutaneous leishmaniasis, but it is usually negative in the diffuse cutaneous form. Antibodies are often slow to develop and of low titer.

Disinfection [108]

Leishmania spp do not remain viable outside a host or *in vitro* culture. They can be inactivated by 1% sodium hypochlorite, 2% glutaraldehyde or formaldehyde. They are also susceptible to heat of 50–60°C.

Treatment [108]

Visceral or cutaneous leishmaniasis can usually be cured in immunocompetent individuals. Pentavalent antimonials can be used where the parasites are sensitive to these drugs, but resistance is a major problem in some areas. Other drugs such as allupurinol, amphotericin B or liposomal amphotericin B, and miltefosine may also be used. Most of the drugs used to treat leishmaniasis must be given parenterally. Visceral leishmaniasis in AIDS patients is often resistant to treatment, and many patients relapse.

Cutaneous leishmaniasis may be treated to speed healing, decrease scarring and decrease the risk of mucosal disease or relapse. Intralesional, topical or systemic drugs may be used, depending on the species of *Leishmania* and the risk of more serious complications. Cryotherapy, thermotherapy, or curettage have also been employed in some cases. Some cutaneous leishmaniasis lesions that are improving may simply be observed, if they are caused by relatively benign organisms. Mucosal leishmaniasis is a serious condition and it is treated with systemic drugs.

Prevention[108]

Preventive measures against sandflies include

- using insect repellents such as DEET
- covering exposed skin
- staying on higher floors of buildings in the evening or at night, as these insects are poor fliers.
- Fans can also be helpful, and insecticidal sprays can be used to kill the insects inside houses.
- Insecticide-treated bed nets decrease bites from these insects at night.
- Insecticide spraying programs have been conducted in some countries.

Treatment of human patients may be helpful in areas where anthroponotic transmission is important. Decreasing the incidence of *L. infantum* in dogs can help protect people from this

organism. Some studies have shown that insecticide impregnated dog collars protected both dogs and children in areas where they were used. Infected dogs have been culled in some countries. The only practical way to decrease the incidence of these disease is personal protection with insect repellents and other measures.

Drugs in lead optimization and preclinical phases[76]

Plant products are an abundant source of leads, evidenced in the area of anti-malarials. Licochalcone A from the Chinese Liquorice plant glycyrrhiza has shown reasonable oral efficacy in experimental models of VL and CL.

Another compound PX-6581 from the Vietnamese plant Maesa balansae showed significant activity in VL models but was not progressed due to toxicity. Isopropyl quinolines isolated from Galipea longiflora in Bolivia also showed activity in VL and CL models and studies on further SAR have recently been published.

Therapeutic switching or "piggy-back chemotherapy" is another potential source for new antileishmanial compounds. Azoles, originally developed as antifungal drugs have shown activity against Leishmania spp. Like fungi, Leishmania synthesize 24-substituted sterols, such as ergosterol (mammals have cholesterol). Azoles can inhibit a key enzyme of this pathway, 14α-demethylase. Ketoconazole, itraconazole and fluconazole have given equivocal results in trials against both CL and VL. Bisphosphonates, used for the treatment of bone disorders such as osteoporosis, are another example of therapeutic switching. Two of these drugs, risedronate and pamidronate, were active against experimental infections of both CL and VL.

6. DRUG PROFILE

Drug name : Ketoconazole [122]

Brand name : Extina, Nizoral, Ketozole, Xolegel, Fungrest, ketoderm, ketozoral

IUPAC name[122] : 1-[4-(4-{[(2R,4S)-2-(2,4-Dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan -4-yl] methoxy}phenyl) piperazin-1-yl] ethan-1-one.

CAS number : 65277-42-1

Formula : $C_{26}H_{28}Cl_2N_4O_4$

Molecular weight : 531.4 g/mol

Physical form : A White to off-white crystalline powder

Odour : Odourless

Melting range : 148°C to 152°C

Structure:

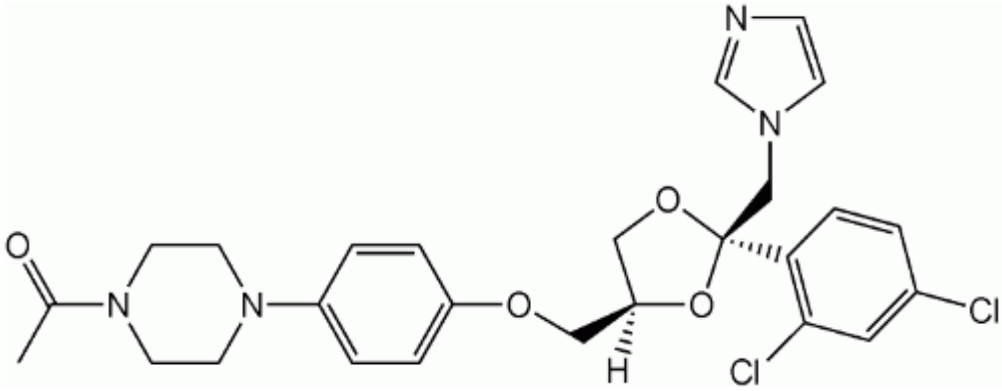


Figure 11. Structure of Ketoconazole.

Solubility[49] : Freely soluble in Dichloromethane, soluble in chloroform and in methanol, sparingly soluble in ethanol (95 percent), practically insoluble in water and in ether.

CLINICAL PHARMACOLOGY

Pharmacokinetics

Mean peak plasma levels of approximately 3.5 μg/ml are reached within 1 to 2 hours, following oral administration of a single 200 mg dose taken with a meal. Subsequent plasma elimination is biphasic with a half-life of 2 hours during the first 10 hours and 8 hours thereafter. Following absorption from the gastrointestinal tract, Ketoconazole is converted into several inactive metabolites. The major identified metabolic pathways are oxidation and degradation of the imidazole and piperazine rings, oxidative dealkylation and aromatic hydroxylation. About 13% of the dose is excreted in the urine of which 2 to 4% is unchanged drug. The major route of excretion is through the bile into the intestinal tract. *In vitro* plasma protein binding is about 99% mainly to the albumin fraction. Only a negligible proportion of Ketoconazole reaches the cerebrospinal fluid. Ketoconazole is a weak dibasic agent and thus requires acidity for dissolution and absorption.

Electrocardiogram

Pre-clinical electrophysiological studies have shown that Ketoconazole inhibits the rapidly activating component of the cardiac delayed rectifier potassium current, prolongs the action potential duration and may prolong the QTc interval. Data from some clinical PK/PD studies and drug interaction studies suggest that oral dosing with Ketoconazole at 200 mg twice daily for 3-7 days can result in an increase of the QTc interval, a mean maximum increase of about 6 to 12 msec was seen at Ketoconazole peak plasma concentrations about 1-4 hours after Ketoconazole administration.

Mechanism of Action

Ketoconazole blocks the synthesis of ergosterol, a key component of the fungal cell membrane, through the inhibition of cytochrome P-450 dependent enzyme lanosterol 14α-demethylase responsible for the conversion of lanosterol to ergosterol in the fungal cell membrane. This results in an accumulation of methylated sterol precursors and a depletion of ergosterol within the cell membrane thus weakening the structure and function of the fungal cell membrane.

Activity *In Vitro & In Vivo*

Ketoconazole is active against clinical infections with *Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum, Paracoccidioides brasiliensis,Leishmania braziliensis.*

CONTRAINDICATIONS

Drug Interactions

Co-administration of a number of CYP3A4 substrates is contraindicated with Ketoconazole. Co-administration with Ketoconazole can cause elevated plasma concentration of these drugs and may increase or prolong both therapeutic and adverse effects. For example, increased plasma concentration of some of these drugs can lead to QTc prolongation and ventricular tachyarrhythmias including occurrence of torsades de pointes, a potentially fatal arrhythmia.

Liver Disease

The use of Ketoconazole is contraindicated in patients with acute or chronic liver disease.

Hypersensitivity It is contraindicated in patients who have shown hypersensitivity to the drug.

WARNINGS

Ketoconazole should be used only when other effective antifungal therapy is not available or tolerated and the potential benefits are considered to outweigh the potential risks.

Hepatotoxicity

Serious hepatotoxicity including cases with a fatal outcome or requiring liver transplantation has occurred with the use of oral Ketoconazole. Some patients had no obvious risk factors for liver disease. Serious hepatotoxicity was reported both by patients receiving high doses for short treatment durations and by patients receiving low doses for long durations. The hepatic injury has usually but not always been reversible upon discontinuation of Ketoconazole treatment. Cases of hepatitis have been reported in children.

At baseline, obtain laboratory tests (such as SGGT, alkaline phosphatase, ALT, AST, total bilirubin (TBL), Prothrombin Time (PT), International Normalization Ratio (INR) and testing for

viral hepatitides). Patients should be advised against alcohol consumption while on treatment. If possible, use of other potentially hepatotoxic drugs should be avoided in patients receiving Ketoconazole .

Prompt recognition of liver injury is essential. During the course of treatment, serum ALT should be monitored weekly for the duration of treatment. If ALT values increase to a level above the upper limit of normal or 30 percent above baseline or if the patient develops symptoms, ketoconazole treatment should be interrupted and a full set of liver tests should be obtained. Liver tests should be repeated to ensure normalization of values. Hepatotoxicity has been reported with restarting oral Ketoconazole (rechallenge). If it is decided to restart oral Ketoconazole monitor the patient frequently to detect any recurring liver injury from the drug.

QT Prolongation and Drug Interactions Leading to QT Prolongation

Ketoconazole can prolong the QT interval. Co-administration of the following drugs with Ketoconazole is contraindicated to dofetilide, quinidine, pimozide, and cisapride. Ketoconazole can cause elevated plasma concentrations of these drugs which may prolong the QT interval, sometimes resulting in life threatening ventricular dysrhythmias such as torsades de pointes.

Adrenal Insufficiency

Ketoconazole Tablets decrease adrenal corticosteroid secretion at doses of 400 mg and higher. This effect is not shared with other azoles. The recommended dose of 200 mg - 400 mg daily should not be exceeded.

Adrenal function should be monitored in patients with adrenal insufficiency or with borderline adrenal function and in patients under prolonged periods of stress (major surgery, intensive care, etc).

Adverse Reactions Associated with Unapproved Uses

Ketoconazole has been used in high doses for the treatment of advanced prostate cancer and for Cushing’s syndrome when other treatment options have failed. The safety and effectiveness of Ketoconazole have not been established in these settings and the use of Ketoconazole for these indications is not approved by FDA.

In a clinical trial involving 350 patients with metastatic prostatic cancer, eleven deaths were reported within two weeks of starting treatment with high doses of Ketoconazole tablets (1200 mg/day). It is not possible to ascertain from the information available whether death was related to Ketoconazole therapy or adrenal insufficiency in these patients with serious underlying disease.

Hypersensitivity

Anaphylaxis has been reported after the first dose. Several cases of hypersensitivity reactions including urticaria have also been reported.

Enhanced Sedation

Co-administration of oral Ketoconazole with oral midazolam, oral triazolam or alprazolam has resulted in elevated plasma concentration of these drugs. This may potentiate and prolong hypnotic and sedative effects, especially with repeated dosing or chronic administration of these agents. Concomitant administration of Ketoconazole tablets with oral triazolam, oral midazolam or alprazolam is contraindicated.

Myopathy

Co-administration of CYP3A4 metabolized HMG-CoA reductase inhibitors such as simvastatin, and lovastatin is contraindicated with Ketoconazole tablets.

PRECAUTIONS

General

Ketoconazole tablets have been demonstrated to lower serum testosterone. Once therapy with Keoconazole tablets has been discontinued, serum testosterone levels return to baseline values. Testosterone levels are impaired with doses of 800 mg per day and abolished by 1600 mg per day. Clinical manifestations of decreased testosterone concentration may include gynecomastia, impotence and oligospermia.

Information for Patients

Patients should be instructed to report any signs and symptoms which may suggest liver dysfunction so that appropriate biochemical testing can be done. Such signs and symptoms may include unusual fatigue, anorexia, nausea and/or vomiting, abdominal pain, jaundice, dark urine or pale stools.

Drug Interactions

Drugs that affect the absorption, distribution, metabolism and excretion of Ketoconazole may alter the plasma concentration of Ketoconazole. For example gastric acid suppressants (e.g., antacids, histamine H2-blockers, proton pump inhibitors) have been shown to reduce plasma concentration of Ketoconazole. Ketoconazole is a substrate and potent inhibitor of CYP3A4. Therefore, the following drug interactions may occur when Ketoconazole is co-administered with other drugs that interact with CYP3A4.

1. Ketoconazole may decrease the elimination of drugs metabolized by CYP3A4, thereby increasing their plasma concentration. Increased exposure to these drugs may cause an increase or prolongation of their therapeutic and/or adverse effects. Concomitant use with Ketoconazole tablets is contraindicated for drugs known to present a risk of serious side effects with increased exposure. For others, monitoring of plasma concentration is advised when possible. Clinical signs and symptoms associated with these drugs should be monitored with dosage adjusted as needed.

2. Inducers of CYP3A4 may decrease the plasma concentration of Ketoconazole. Ketoconazole may not be effective in patients concomitantly taking one of these drugs. Therefore, administration of these drugs with Ketoconazole is not recommended.

3. Other inhibitors of CYP3A4 may increase the plasma concentration of Ketoconazole. Patients who must take Ketoconazole concomitantly with one of these drugs should be monitored closely for signs or symptoms of increased or prolonged pharmacological effects of Ketoconazole.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Ketoconazole did not show any signs of mutagenic potential when evaluated using the dominant lethal mutation test or the *Ames Salmonella* microsomal activator assay. Ketoconazole was not carcinogenic in an 18-month, oral study in Swiss albino mice or a 24-month oral carcinogenicity study in Wistar rats at dose levels of 5, 20 and 80 mg/kg/day. The high dose in these studies was approximately 1times the clinical dose in humans for mouse or 2 times the clinical dose in humans for rats based ona mg/m^2 comparison.

Pregnancy

Teratogenic effects: *Pregnancy Category C:* Ketoconazole has been shown to be teratogenic (syndactylia and oligodactylia) in the rat when given in the diet at 80 mg/kg/day (2 times the maximum

recommended human dose, based on body surface area comparisons). However, these effects may be related to maternal toxicity, evidence of which also was seen at this and higher dose levels.

There are no adequate and well controlled studies in pregnant women. Ketoconazole tablets should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nonteratogenic Effects

Ketoconazole has also been found to be embryotoxic in the rat when given in the diet at doses higher than 80 mg/kg during the first trimester of gestation. In addition, dystocia (difficult labor) was noted in rats administered with oral Ketoconazole during the third trimester of gestation. This occurred when Ketoconazole was administered at doses higher than 10 mg/kg (about one fourth the maximum human dose, based on body surface area comparison).

Nursing Mothers

Ketoconazole has been shown to be excreted in the milk. Mothers who are under treatment with Ketoconazole tablets should not breast feed.

Pediatric Use

Ketoconazole Tablets have not been systematically studied in children of any age and essentially no information is available on children under 2 years. Ketoconazole tablets should not be used in pediatric patients unless the potential benefit outweighs the risks.

ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

The following adverse reactions were reported in clinical trials:

- Immune System Disorders: anaphylactoid reaction.
- Endocrine Disorders: gynecomastia.
- Metabolism and Nutrition Disorders: alcohol intolerance, anorexia, hyperlipidemia, increased appetite.
- Psychiatric Disorders: insomnia, nervousness.
- Nervous System Disorders: headache, dizziness, paresthesia, somnolence.
- Eye Disorders: photophobia;

- Vascular Disorders: orthostatic hypotension
- Respiratory disorders:

  Thoracic and Mediastinal Disorders: epistaxis;
- Gastrointestinal Disorders: vomiting, diarrhea, nausea, constipation, abdominal pain, abdominal pain upper, dry mouth, dysgeusia, dyspepsia, flatulence, tongue discoloration;
- Hepatobiliary Disorders: hepatitis, jaundice, hepatic function abnormal;
- Skin and Subcutaneous Tissues Disorders: erythema multiforme, rash, dermatitis, erythema, urticaria, pruritus, alopecia, xeroderma.
- Musculoskeletal and Connective Tissue Disorders: myalgia;
- Reproductive System and Breast Disorders: menstrual disorder; General Disorders and Administration Site Conditions: asthenia, fatigue, hot flush, malaise, edema peripheral, pyrexia, chills;
- Investigations: platelet count decreased.

OVERDOSAGE

In the event of acute accidental overdose, treatment consists of supportive and symptomatic measures. Within the first hour after ingestion, activated charcoal may be administered.

DOSAGE AND ADMINISTRATION

There should be laboratory as well as clinical documentation of infection prior to starting Ketoconazole therapy. The usual duration of therapy for systemic infection is 6 months. Treatment should be continued until active fungal infection has subsided.

Adults

The recommended starting dose of (Ketoconazole) tablets is a single daily administration of 200 mg (one tablet). If clinical responsiveness is insufficient within the expected time, the dose may be increased to 400 mg (two tablets) once daily.

Children

In small number of children over 2 years of age, a single daily dose of 3.3 to 6.6 mg/kg has been used. Ketoconazole tablets have not been studied in children under 2 years of age.

INDICATIONS AND USAGE

Ketoconazole should be used only when other effective antifungal therapy is not available or tolerated and the potential benefits are considered to outweigh the potential risks.

It is indicated for the treatment of the following systemic fungal infections in patients who have failed or who are intolerant to other therapies: blastomycosis, coccidioidomycosis, histoplasmosis, chromomycosis, and paracoccidioidomycosis. Ketoconazole should not be used for fungal meningitis because it penetrates poorly into the cerebrospinal fluid.

Orally administered Ketoconazole is effective in dermatophytosis because it is concentrated in the stratum comeum; is an altemative to griseofulvin, but use is restricted due to potential adverse effects. Though effective in monilial vaginitis, oral therapy (for 5-7 days) with Ketoconazole is reserved for recurrent cases or those not responding to topical agents.

Systemic mycosis: Administered orally, Ketoconazole is effective in several types of systemic mycosis, but itraconazole and fluconazole, being more active with fewer side effects, have largely replaced it for these indications except for considerations of cost. Ketoconazole is occasionally used in dermal leishmaniasis and kala azar. High-dose Ketoconazole has been used in Cushing's syndrome to decrease corticosteroid production. [123]

7. EXCIPIENT PROFILE

SORBITAN MONOLAURATE 20[125-127]

SYNONYM

Arlacel 20; Emsorb 515; SPAN(R) 20; Arlasel 20; Dehymuls SML; E493; Glycomul L;

Hodag SML; Liposorb L; Montane 20; Protachem SML; Sorbester P12;

Sorbirol L; sorbitan laurate; Span 20; Tego SML

IUPAC NAME[127]

[(2R) – 2- [(3R,4S)-3,4- dihydroxyoxolan–2–yl]-2-hydroxy ethyl] dodecanoate.

EMPIRICAL FORMULA

$C_{18}H_{34}O_6$

MOLECULAR WEIGHT

346.47 g/mol

STRUCTURE

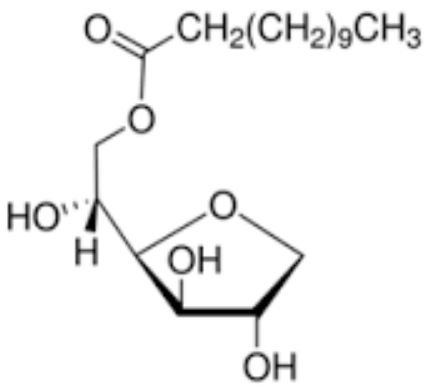


Figure 12 Sorbitan monolaurate

DESCRIPTION

Amber to brown oily liquid.

PROPERTIES

- Density – 1.032 g/ml at 25° C.(lit.)
- Hydroxyl value- 330-358 mg KOH/g
- Saponification value- 158-170 mg KOH/g
- Acid value ≤ 1.5%
- Melting point- >200°C
- Boilingpoint 516.1°C at 760 mmHg.
- Flash point – 176.9°C
- HLB value- 8.6
- Solubility – slighty soluble in isopropanol, tetracarp,xylene, cotton seed oil and mineral oil. Slightly soluble in liquid paraffin and insoluble in water.

FUNCTIONAL CATEGORY

- Emulsifying agent
- Non- ionic surfactant
- Lubricant
- Wetting agent
- Dispersing/ Suspending agent

STORAGE

- It should be stored in a well- closed container in a cool, dry place.

SORBITAN MONOPALMITATE[48]

SYNONYMS

Arbunol S-40; Alracel 40; Crill 2; Liposorb P; Montane 40; Sorbitan palmitate; Span 40.

CHEMICAL NAME

Sorbitan Mono Hexadecanoate

EMPIRICAL FORMULA

$C_{22}H_{42}O_6$

MOLECULAR WEIGHT

403

STRUCTURE

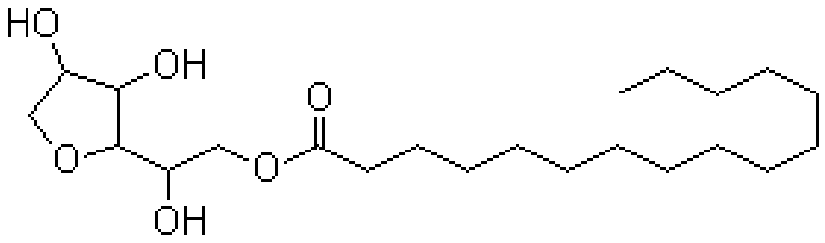


Figure 13 Sorbitan Monopalmitate

DESCRIPTION

Creamy solid

PROPERTIES

- Acid value- 3- 7
- Hydroxyl value- 270- 303
- Saponification value- 142- 152
- Melting point- 43- 48°C

- Density (g/cm^3)- 1.0 g/cm^3
- HLB value- 6.7

FUNCTIONAL CATEGORY

- Emulsifying agent
- Non- ionic surfactant
- Solubiizing agent
- Wetting agent
- Dispersing/ Suspending agent

STABILITY

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

STORAGE

It should be stored in a well- closed container in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25 mg/ kg body weight.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

SORBITAN MONOSTEARATE[48]

SYNONYM

Arbunol S- 60; Alracel 60; Crill 3; Liposorb S-K; Montane 60; Sorbitan stearate; Span 60; Tego SMS.

CHEMICAL NAME

Sorbitan Mono- Octadecanoate

EMPIRICAL FORMULA

$C_{24}H_{46}O_6$

MOLECULAR WEIGHT

431

STRUCTURE

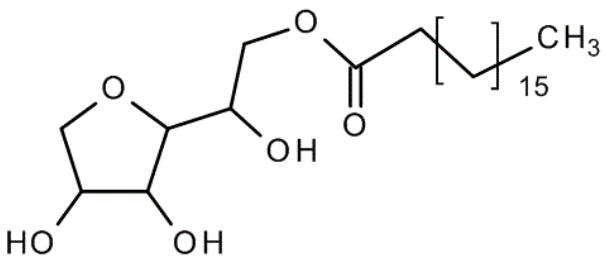


Figure 14 Sorbitan monostearate

DESCRIPTION

Cream solid

PROPERTIES

- Acid value- 5- 10
- Hydroxyl value- 235- 260
- Saponification value- 147- 157
- Melting point- 53- 57°C
- HLB value- 4.7

FUNCTIONAL CATEGORY

- Emulsifying agent
- Non- ionic surfactant
- Solubilizing agent
- Wetting agent
- Dispersing/ Suspending agent

STABILITY

- Gradual soap formation occurs with strong acids or bases
- Stable in weak acids or bases.

STORAGE

It should be stored in a well- closed container in a cool, dry place.

SAFETY

Daily intake according to the WHO limit is about 25 mg/ kg body weight.

HANDLING PRECAUTIONS

Eye protection and Gloves are recommended.

CHOLESTEROL[48]

SYNONYM

Cholesterolum; Cholesterin

CHEMICAL NAME

Cholest- 5-en-3β- ol

EMPIRICAL FORMULA

$C_{27}H_{46}O$

MOLECULAR WEIGHT

386.67

STRUCTURE

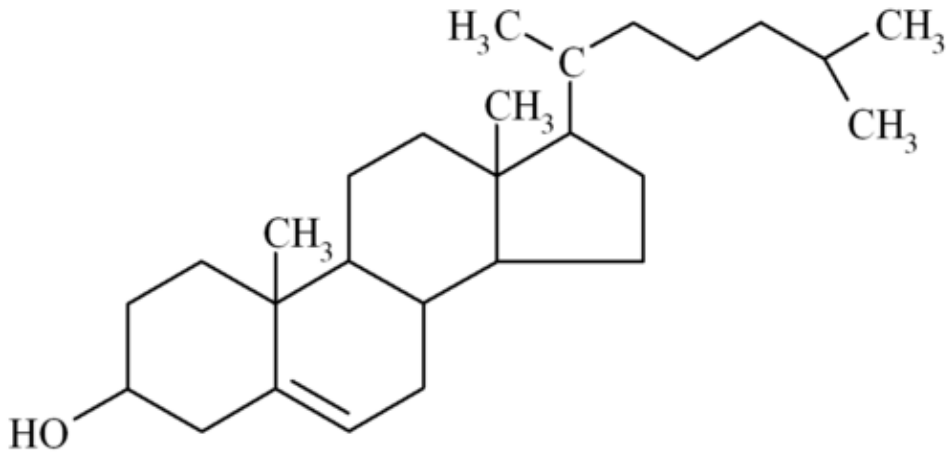


Figure 15. Cholesterol

FUNCTIONAL CATEGORY

- Emulsifying agent
- Emollient

DESCRIPTION

- Cholesterol occurs as white or faintly yellow, almost odourless, pearly leaflets, needles, powder or granules.
- On prolonged exposure to light and air, it acquires a yellow to tan colour.

PROPERTIES

- Boiling point- 360°C
- Density- 1.052 g/ cm^3 for anhydrous form
- Melting point- 147- 150°C
- Solubility – soluble in acetone and vegetable oils, Practically insoluble in water, Soluble in chloroform: methanol mixture.

STABILITY AND STORAGE CONDITIONS

It is stable, and should be stored in a well- closed container and protected from light.

SAFETY

It is generally regarded as an essentially non- toxic and non- irritant material at the levels employed as an excipients.

HANDLING PRECAUTIONS

Rubber or plastic gloves, eye protection and a respirator are recommended.

CARBOPOL 940[128,129,130.]

NON-PROPRIETARY NAME:

BP- Carbomers, PhEur- Carbomera, USPNF- Carbomer.

SYNONYMS:

Acritamer; acrylic acid polymer; Carbopol; Carboxy Polymethylene; Polyacrylic acid; Carboxyvinyl polymer; Pemulen; Ultrez.

CHEMICAL NAME

Poly (acrylic acid)

IUPAC NAME

Prop-2- enoic acid

EMPIRICAL FORMULA

$(C_3H_4O_2)n$

MOLECULAR WEIGHT

Variable. For Carbopol 940, M.W = 940.

CAS NUMBER: 9003-01-4

STRUCTURE

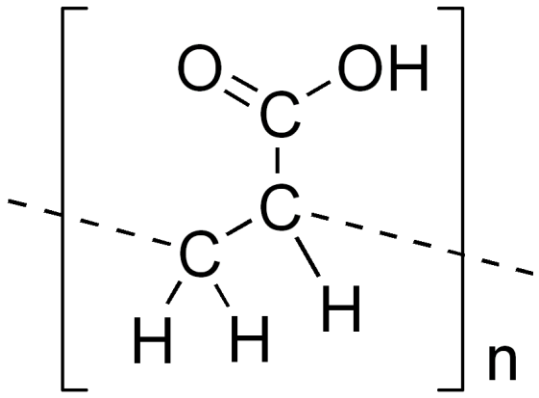


Figure 16. carbopol

FUNCTIONAL CATEGORY

- Bioadhesive, Emulsifying agent, release-modifying agent, suspending agent, tablet-binder, viscosity increasing agent.
- Carbopol 940 polymer is an extremely efficient rheology modifier capable of providing high viscosity and forms sparkling clear water or hydro-alcoholic gels and creams.

DESCRIPTION

- Carbomers are white-colored, 'fluffy', acidic, hygroscopic powders with a slight characteristic odour.

PROPERTIES

- Boiling point- 116°C.
- Density- 1.2 g/ ml at 25°C.
- Flash point: 100°C
- Melting point- 12.5°C.[129]
- Solubility – soluble in water and ethanol.

STABILITY AND STORAGE CONDITIONS

It is hygroscopic, and should be stored in a well- closed container and protected from moisture.

SAFETY STATEMENT

Moderately toxic by ingestion. when heated to decomposition it emits acrid smoke and irritating vapors.

8. MATERIALS AND METHODS

The list of drug, excipients used and their manufacturer are show in Table

Table 1: List of Materials Used

S. No.	DRUG/ EXCIPIENT	MANUFACTURER/ SUPPLIER
1	Ketoconazole USP	East West Pharma, Haridwar, Uttarakhand-247661.
2	Cholesterol	S.D. Fine chem. Ltd
3	Sorbitan Monopalmitate	SPAK Orgochem India Pvt Ltd
4	Sorbitan Monostearate	SPAK Orgochem India Pvt Ltd
5	Sorbitan Monolaurate	Lab chemicals Ltd
6	Carbopol 940	East West Pharma, Haridwar, Uttarakhand-247661.
6	Sodium chloride	Indian research products
7	Potassium dihydrogen ortho phosphate	Nice chemicals Pvt Ltd
8	Disodium hydrogen ortho phosphate	Indian research products
9	Chloroform	Merck specialities Pvt Ltd
10	Methanol	Sisco research laboratories Pvt Ltd
11	n-propanol	Lab Chemicals Ltd
12	Dialysis Membrane 50	Himedia

The list of equipments used in the study and their manufacturer are shown in Table

Table 2: List of Equipments

S.No	EQUIPMENTS	MANUFACTURER
1	Rotary flash evaporator	Equitron
2	Ultra Sonicator	Lark
3	Electronic balance	Model No.- Jewel- 3 Eagle
4	Magnetic stirrer with hot plate	Eltek Magnetic Stirrers
5	UV- Visible Spectrophotometer	SHIMADZU UV- 1800
6	High speed Cooling centrifuge	Remi
7	Binocular microscope	Olympus
8	Malvern zeta sizer	Malvern, Germany
9	Scanning electron microscope	Hitachi, Japan
10	FT- IR Spectrophotometer	Nicolet, India
11	Refrigerator	Whirlpool
12	BOD incubator	MC Dalal

METHODOLOGY

PREPARATION OF PHOSPHATE BUFFERED SALINE (PBS) pH 7.4[49]

2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride were dissolved in sufficient quantity of distilled water and the volume made up to 1000 ml.

DRUG EXCIPIENT COMPATIBILITY STUDIES[50,117]

The possibilities of drug- excipients (cholesterol, nonionic surfactant) interactions were investigated by FT- IR spectrum study. The FT- IR spectrum of pure drug and combination of drug with excipients were recorded.

The spectrum was recorded in the wavelength region of 4000 to 400 cm^{-1}.

STANDARD CURVE FOR KETOCONAZOLE[50]

100 mg of Ketoconazole was accurately weighed and dissolved in a small quantity of methanol and made up to 100 ml with methanol. From this primary solution, 10 ml was pipetted out and made up to 100 ml with phosphate buffered saline pH 7.4. From this secondary solution aliquots were taken to produce concentration of 2, 4, 6, 8, 10 µg/ ml. The absorbance of the resulting solution was measured at 225 nm in the UV- Visible Spectrophotometer (Shimadzu) using phosphate buffered saline pH 7.4 with little methanol as blank. The standard curve was plotted taking concentration in X- axis and absorbance in Y- axis.

FORMULATION OF KETOCONAZOLE NIOSOMES[35, 50,100]

The niosomal formulations were prepared by thin film hydration technique. Accurately weighed quantities of drug, non- ionic surfactant (Span 20, 40, 60) and cholesterol were dissolved in sufficient quantity of solvent mixture (Chloroform: Methanol 2:1) to give a clear solution. The resulting solution is poured into a 1000 ml rotary flask and evaporated under vacuum (20- 25mm Hg) at 60°±2°C with the rotation speed of 100 rpm to form a uniform thin dry film. The rotary flask was removed from the bath and allowed to return to room temperature. The thin film formed was hydrated with 20 ml of distilled water while rotating

the flask at 50 rpm (gentle agitation) at a temperature 60°±2°C. The resulting niosomal suspension was stored in a tightly closed container in a refrigerator.

Table 3: Formulation Code of Niosomes

FORMULATION CODE	NON- IONIC SURFACTANT	DRUG: SURFACTANT: CHOLESTEROL (m moles)
KTZ 20- 1	SPAN 20	1:1:1
KTZ 20- 2		1:2:1
KTZ 20- 3		1:3:1
KTZ 20- 4		1:4:1
KTZ 20- 5		1:5:1
KTZ 40- 1	SPAN 40	1:1:1
KTZ 40- 2		1:2:1
KTZ 40- 3		1:3:1
KTZ 40- 4		1:4:1
KTZ 40- 5		1:5:1
KTZ 60- 1	SPAN 60	1:1:1
KTZ 60- 2		1:2:1
KTZ 60- 3		1:3:1
KTZ 60- 4		1:4:1
KTZ 60- 5		1:5:1

In the Drug: Surfactant: Cholesterol ratio, 1 stands for 25 µmol.

OPTIMIZATION OF PROCESS- RELATED VARIABLES

Effect of hydration time[100, 131]

The niosomal formulations containing Span 40 at different ratios and a fixed amount of cholesterol (1:1:1, 1:2:1) were hydrated with 10 ml of distilled water for 30 minutes, 60 minutes and 90 minutes. The vesicle formation and entrapment efficiency of the formulations were calculated by centrifugation method.

Effect of capacity and rotational speed of evaporator flask[100, 131]

The thickness and uniformity of the film depends upon the rotational speed of the evaporator flask. The niosomal formulations were subjected to various speeds i.e. 50 rpm, 100 rpm and 150 rpm. The appearance of the film was checked by visual observation.

Effect of Sonication time[100, 131]

The Niosomal formulations containing Span 40 at different ratios and a fixed amount of Cholesterol (1:1, 2:1, 3:1, 4:1, 5:1) were subjected to ultrasonic vibration using Ultrasonicator. To study the effect of sonication time, the formulations were subjected to sonication for various time intervals (1 min, 2 mins, 3 mins, 4 mins and 5 mins). The entrapment efficiency of the formulations were measured.

Effect of osmotic shock [23,100]

The effect of osmotic shock on niosomal formulations was investigated by monitoring the change in vesicle diameter after incubation of niosomal suspension in media of different tonicity i.e., 1.6% NaCl (hypertonic), 0.9% NaCl (isotonic) and 0.5% NaCl (hypotonic). Suspensions were incubated in these media for 3 hours and the change in vesicle size was measured by optical microscopy with a calibrated eyepiece micrometer.

CHARACTERIZATION OF NIOSOMES

PARTICLE SIZE ANALYSIS[45,100]

The Niosomal suspension was diluted, filled in a cuvette using suitable blank and the average vesicle size of the Niosomes was determined using Malvern zeta sizer.

DRUG CONTENT ANALYSIS[6,50,100]

The amount of drug in the formulation was determined by lysing the niosomes using 50% n-propanol. 1 ml of the niosomal preparation was pipetted out, sufficient quantity of 50% n-propanol was added and shaken well for the complete lysis of the vesicles. After suitable dilution with the phosphate buffered saline of pH 7.4 containing 10% Methanol, the absorbance of the solution was measured at 225 nm in the UV- Visible Spectrophotometer. The excipients mixture without the drug treated in the similar manner as the niosomal suspension was used as blank. The drug content was calculated.

ESTIMATION OF ENTRAPMENT EFFICIENCY[100]

The entrapment efficiency of the formulations was determined by centrifuging 1 ml of the suspension diluted to 10 ml with distilled water at 15,000 rpm for 60 minutes at 4°C using a high speed cooling centrifuge in order to separate niosomes from unentrapped drug. The free drug concentration in the supernatant was determined at 225 nm using UV- Visible Spectrophotometer after suitable dilution. The percentage of drug entrapment in niosomes was calculated using the following formula,

$$\% \text{ drug entrapment} = \frac{(\text{Total drug} - \text{Drug in supernatant liquid})}{\text{Total drug}} \times 100$$

***IN- VITRO* RELEASE STUDY[33, 50]**

In- vitro drug release pattern was studied using dialysis membrane. The niosomal preparation after separation of unentrapped drug was placed in an open ended glass tube, one end of which was tied with the dialysis membrane. This acted as the donor compartment. Then the open ended tube was placed in a beaker containing 100 ml phosphate buffered saline pH 7.4, which acted as receptor compartment. The temperature of the receptor medium was maintained at 37°±2°C and the medium was agitated at a speed of 100 rpm using a magnetic stirrer.

5 ml of the samples were collected at a predetermined time and replenished immediately with the same volume of fresh buffer PBS pH 7.4. The sink condition was

maintained throughout the experiment. The collected samples were analyzed Spectrophotometrically at 225 nm using UV- Visible spectrophotometer.

KINETICS OF DRUG RELEASE[100,131]

To study the kinetics and mechanism of drug release, the release data of the *in- vitro* dissolution study of niosomes were fitted in various kinetic models.

- **Zero order equation**

The zero order release kinetics can be obtained by plotting cumulative percentage of drug release Vs time (hours). It is ideal for the formulation to have release profile of zero order to achieve pharmacological prolonged action.

$$C = K_0 t$$

Where, K_0= Zero order constant in conc/ time

t= time in hours

- **First order equation**

The graph was plotted as log % cumulative drug remaining Vs time in hours.

$$\text{Log } C = \text{Log } C_0 - Kt/2.303$$

Where, C_0= Initial drug concentration

K= First order constant

T= Time in hours

- **Higuchi kinetics**

The graph was plotted with % cumulative drug released Vs square root of time.

$$Q = Kt^{1/2}$$

Where, **K**= constant reflecting design variable system (Differential rate constant)

T= time in hours

The release rate of the drug is inversely proportional to the square root of time and is related to the rate of drug diffusion.

❖ **Hixson and Crowell erosion equation**

To evaluate the drug release with changes in the surface area and the diameter of particles, the data were plotted using the Hixson and Crowell erosion equation. The graph was plotted by cube root of % drug remaining Vs Time in hours.

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC} \times t$$

Where, Q_t= amount of drug released at time t

Q_0= initial amount of drug

K_{HC}= rate constant for Hixson Crowell equation

❖ **Korsmeyer- Peppas equation**

To evaluate the mechanism of drug release, it was further plotted in Peppas equation as log cumulative % of drug released Vs log time.

$$M_t / M_\alpha = Kt^n$$

Where, M_t / M_α= fraction of drug released at time t

T= release time

K= release rate constant

n= diffusional exponent indicative of the mechanism of drug release.

The diffusion mechanism is given by the slope (n) value,

n value	Mechanism
0.45	Fickian diffusion
0.45<n<0.89	Anamolous or non- Fickian diffusion
0.89	Case- II transport
n> 0.89	Super case- II transport

The models were used to analyze the release of pharmaceutical polymeric dosage forms when the release mechanism was not known or more than one type of release was involved. The r^2 and K values were calculated for the linear curve obtained by regression analysis of the above plots.

SCANNING ELECTRON MICROSCOPY[50, 132]

The sizes of the vesicles were measured by scanning electron microscope (HITACHI S- 150). A small amount of sample of niosomes suspension was taken in cover slip on the specimen stub. It was coated with carbon and then with gold vapour using Hitachi vacuum evaporator, model HITACHI S 5 GB. The samples were examined under scanning electron microscope, which is operated at 15 kilovolts and then photographed.

ZETA POTENTIAL ANALYSIS[46]

Zeta potential analysis was used to measure the stability of niosome by studying its colloidal property. Aggregation is attributed to the shielding of the vesicle surface charge by ions in solution and thereby reducing the electrostatic repulsion. Vesicle surface charge can be estimated by measurement of particle electrophoretic mobility and is expressed as the Zeta potential. The study was conducted using Malvern Zeta Analyzer.

FORMULATION OF NIOSOME ENTRAPPED KETOCONAZOLE GEL[50]

The Promising niosomal suspension ,(formulation of niosomes prepared using the optimized ratio of surfactants) containing ketoconazole equivalent to 2% w/w was incorporated into the gel base composed of Carbopol 940 (1.5%), Glycerol (10%), Triethanolamine (q.s.) and distilled water up to 15g.

EVALUATION OF NIOSOMAL GEL[50]

Physical Appearance

The prepared gel was examined for clarity, color, homogeneity and the presence of foreign particles.

pH

2.5g of gel was accurately weighed and dispersed in 25 ml of distilled water. The pH of the dispersion was measured by using a digital pH meter.

Rheological study

Viscosity measurement:

Viscosity was determined by Brookfield programmable DV III ultra viscometer. In the present study, spindle no. CP 52 with an optimum speed of 0.01 rpm was used to measure the viscosity of the preparation.

Content Uniformity: [50]

The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of gel equivalent to 10mg of the drug in 100 ml volumetric flask and suitable volume of 50% n-propanol for lysis of the vesicles. The volume was made up to 100 ml with methanol. The content was filtered through Whatman filter paper No.41. 5 ml of above solution was taken into a 50ml volumetric flask and volume was made upto mark with methanol. The content of Ketoconazole was determined at 225 nm against blank by using the Shimadzu UV/visible spectrophotometer.

Estimation of entrapment efficiency[100]

The entrapment efficiency of the formulations was determined by centrifuging 0.5 g of the gel equivalent to 10mg of ketoconazole diluted to 10 ml with distilled water at 15,000 rpm for 60 minutes at 4°C using a high speed cooling centrifuge in order to separate niosomes from unentrapped drug. The free drug concentration in the supernatant was determined at 225 nm using UV- Visible Spectrophotometer after suitable dilution. The percentage of drug entrapment in niosomes was calculated using the following formula,

$$\% \text{ drug entrapment} = \frac{(\text{Total drug} - \text{Drug in supernatant liquid})}{\text{Total drug}} \times 100$$

***In-vitro* Drug Diffusion study[33]**

In- vitro drug diffusion study was studied using dialysis membrane. The niosomal gel equivalent to 10mg of the drug was placed in an open ended glass tube, one end of which was tied with the dialysis membrane. This acted as the donor compartment. Then the open ended tube was placed in a beaker containing 100 ml phosphate buffered saline pH 7.4, which acted as receptor compartment. The temperature of the receptor medium was maintained at 37°±2°C and the medium was agitated at a speed of 100 rpm using a magnetic stirrer.

5 ml of the samples were collected at a predetermined time and replenished immediately with the same volume of fresh buffer PBS pH 7.4. The sink condition was maintained throughout the experiment. The collected samples were analyzed Spectrophotometrically at 225 nm using UV- Visible spectrophotometer.

STABILITY STUDIES[25, 100]

The stability studies of the optimized niosomal formulations were performed at different conditions of temperature and the effect on physical characterisitics, entrapment efficiency and drug content was noted. The niosomal dispersions were kept in the air tight containers and stored at 2- 8°C and at room temperature (30±2°C) for 30 days and 2 ml samples were withdrawn every 15 days and at the end of 45 days. The samples were analyzed spectrophotometrically at λ_{max} 225 nm after disrupting the vesicles with 50% n-propanol.

9. RESULTS AND DISCUSSIONS

DRUG EXCIPIENT COMPATIBILITY STUDIES

The possibilities of drug- excipient (cholesterol, nonionic surfactant) interactions were investigated by recording the FT- IR spectrum. The FT- IR spectra of the drug and the formulations KTZ 20-3, KTZ 40-4 and KTZ 60-5 are shown in figures, tables.

FT- IR SPECTRA OF KETOCONAZOLE AND THE NIOSOMAL FORMULATIONS

Fig. 17: FT- IR spectra of Ketoconazole

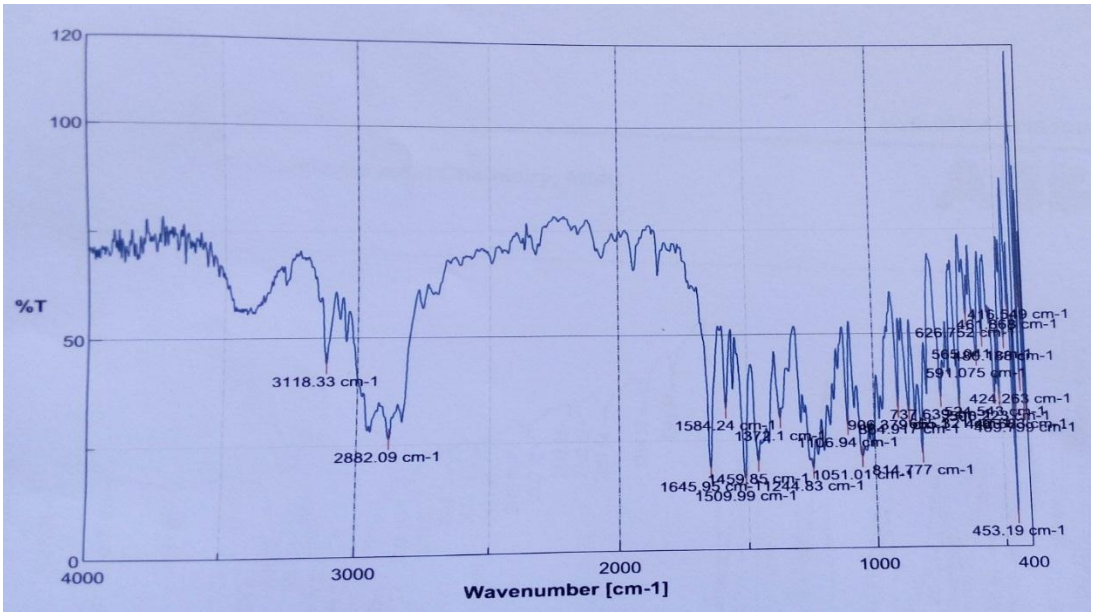


Table 4: Interpretation:

S.No.	WAVE NUMBER (cm^{-1})	CHARACTERISTICS
1	2884	-CH stretching(Aliphatic)
2	1459	-CH bending (Aliphatic)
3	3056	-C- H stretching(Aromatic)
4	1720	-C=O stretching (ketone)
5	735	-C-Cl stretching (substituted Chloride)

Fig. 18: FT- IR spectra of Ketoconazole with Span 20

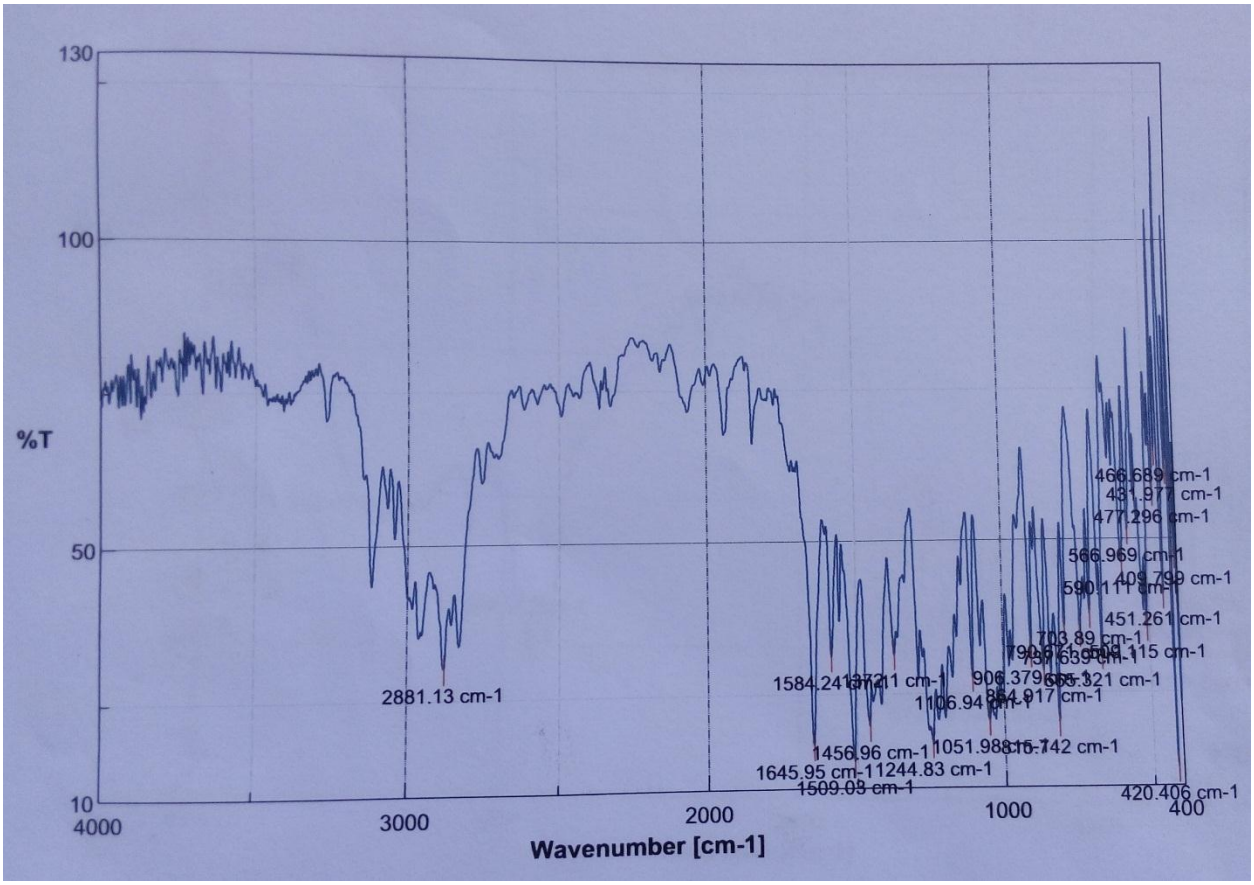


Table 5: Interpretation:

S.No.	WAVE NUMBER (cm^{-1})	CHARACTERISTICS
1	2881	-CH stretching(Aliphatic)
2	1456	-CH bending (Aliphatic)
3	3056	-C- H stretching(Aromatic)
4	1721	-C=O stretching (ketone)
5	737	-C-Cl stretching (substituted Chloride)

It is confirmed that there is no major shifting as well as loss of functional peaks between the spectra of drug and drug loaded niosomes.

Fig. 19: FT- IR spectra of Ketoconazole with Span 40

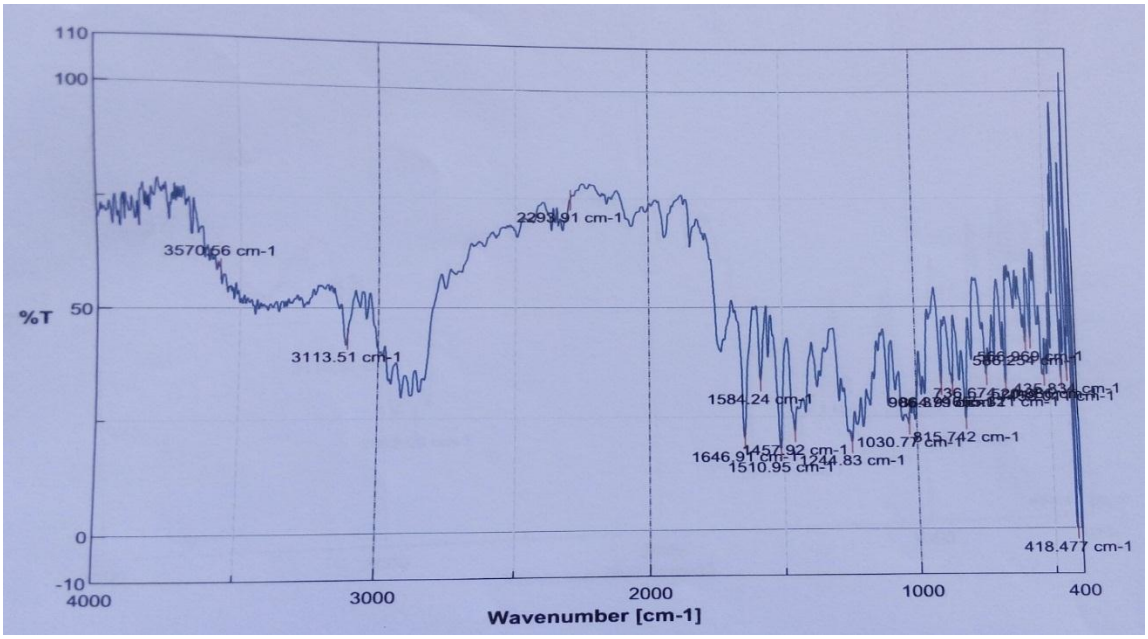


Table 6: Interpretation:

S.No.	WAVE NUMBER (cm^{-1})	CHARACTERISTICS
1	2891	-CH stretching(Aliphatic)
2	1457	-CH bending (Aliphatic)
3	3042	-C- H stretching(Aromatic)
4	1723	-C=O stretching (ketone)
5	736	-C-Cl stretching (substituted Chloride)

It is confirmed that there is no major shifting as well as loss of functional peaks between the spectra of drug and drug loaded niosomes.

Fig. 20: FT- IR spectra of Ketoconazole with Span 60

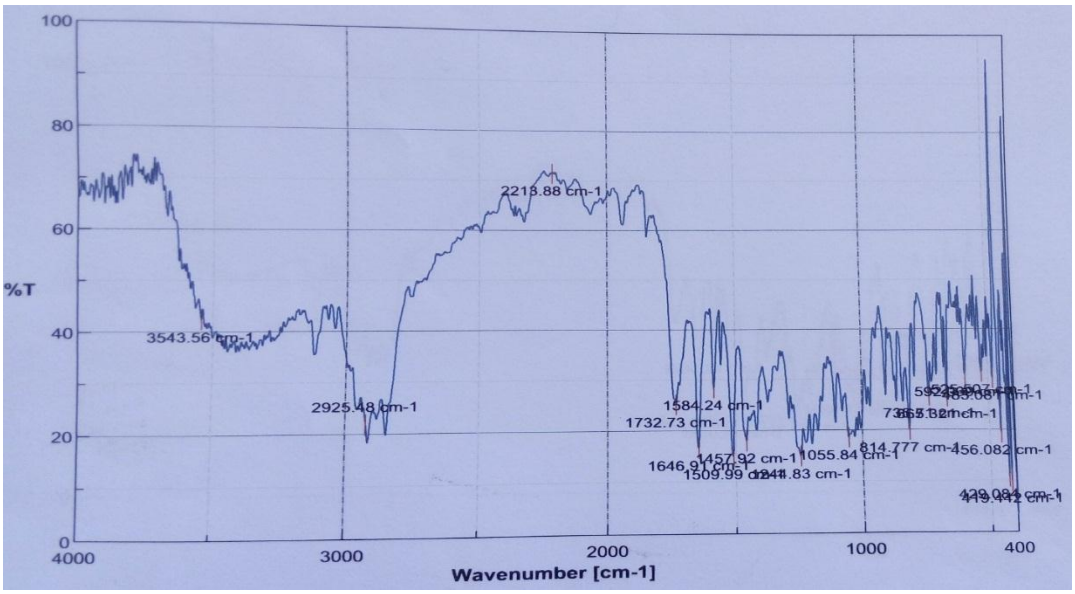


Table 7: Interpretation:

S.No.	WAVE NUMBER (cm^{-1})	CHARACTERISTICS
1	2925	-CH stretching(Aliphatic)
2	1457	-CH bending (Aliphatic)
3	3042	-C- H stretching(Aromatic)
4	1732	-C=O stretching (ketone)
5	736	-C-Cl stretching (substituted Chloride)

It is confirmed that there is no major shifting as well as loss of functional peaks between the spectra of drug and drug loaded niosomes.

STANDARD CURVE FOR KETOCONAZOLE[50]

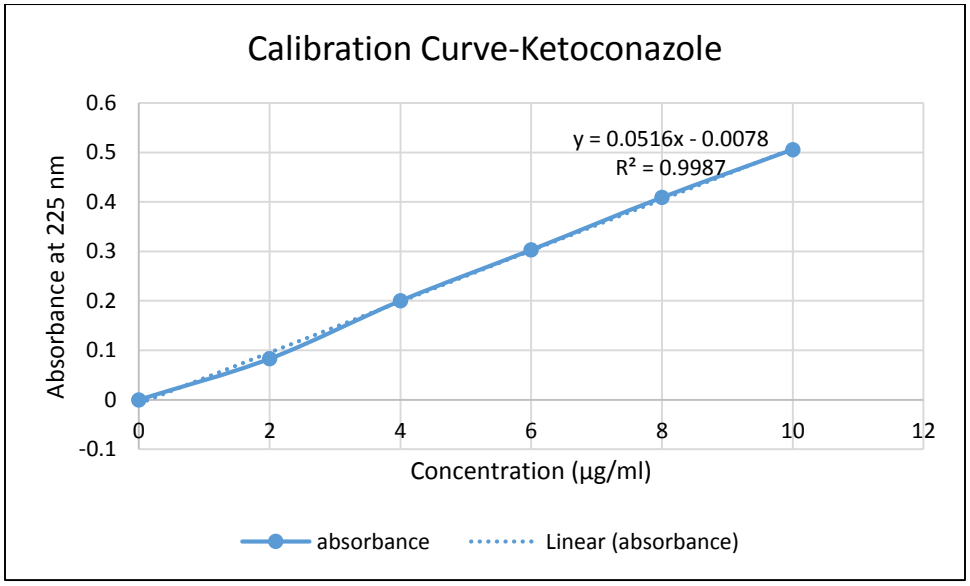
The UV Spectrophotometric method was used to analyze Ketoconazole. The absorbance of the drug in phosphate buffered saline pH 7.4 with small amount of methanol was measured at a wavelength of 225 nm. The results are given in Table and Figure

Table 8: Data for Calibration Curve of Ketoconazole

S. No.	Concentration (µg/ ml)	Absorbance at 286 nm
1	2	0.083 ± 0.0180
2	4	0.200 ± 0.0314
3	6	0.303 ± 0.035
4	8	0.409 ± 0.0396
5	10	0.506 ± 0.0467

*Mean± SD (n=3)

Fig 21: Standard Curve of Ketoconazole in PBS pH 7.4



The standard curve of Ketoconazole in PBS pH 7.4 was linear in 2 to 10 mcg/ ml concentrations, starting from origin. The curve obeys Beer Lambert's law.

OPTIMIZATION OF PROCESS RELATED VARIABLES

❖ **Effect of hydration time**

Table 9: Effect of Hydration Time On Entrapment Efficiency

S. No.	Hydration Time (minutes)	Entrapment Efficiency (%w/w)
1	30	31.06 ± 2.52
2	45	39.5 ±1.15
3	60	75.2 ± 0.16
4	75	73.53 ± 1.5
5	90	71.27 ±1.11

*Mean± SD (n=3)

The niosomal formulations were hydrated with 20 ml of distilled water for 30, 45, 60, 75 and 90 minutes. The parameters like vesicle formation and entrapment efficiency was studied. The results indicated that vesicles were not formed properly but resulted in aggregates with hydration time of 30 minutes. On increase in hydration time, the spherical vesicles were formed and the entrapment efficiency also increased with increase in hydration time from 30 minutes to 60 minutes. Further increase in the hydration time to 90 minutes did not produce any great impact on the entrapment efficiency[100]

❖ **Effect of capacity and rotational speed of evaporator flask[100]**

Thin film was not formed with 50 ml evaporator flask, and the resulting suspension was not uniform. The procedure was repeated with 1000ml evaporator flask. The thickness and uniformity of the film depended on the rotational speed of the flask. A speed of 100 rpm produced a uniform thin film which on hydration produced spherical vesicles. Lower speed (50 rpm) resulted in a non- uniform film and higher speed (150 rpm) produced thick film which on hydration formed aggregates of vesicles. Optimum rotational speed for formation of uniform thin film in a 1000ml flask was found to be 100 rpm initially, which is increased to 150 rpm when 80 % of the solvent has evaporated.

❖ **Effect of Sonication time[100]**

Table. 10:

S. No.	Sonication Time (minutes)	Entrapment Efficiency (%w/w)
1	0	46.06 ± 0.124
2	05	51.13 ±0.016
3	10	60.15 ± 0.040
4	15	57.20 ± 0.081
5	20	55.20 ±1.11

*Mean± SD (n=3)

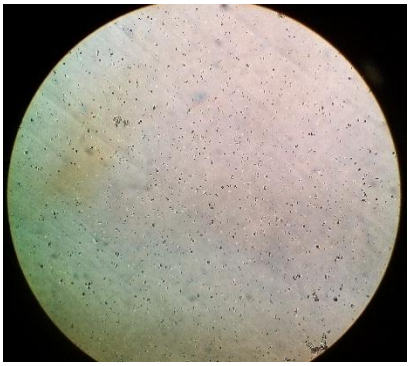
Formulations were sonicated three times for 5 minutes with 2 sec running time and 1 sec interval. Spherical vesicles were not observed after 10 minutes of sonication. The entrapment efficiency decreased as sonication time increased above 10 minutes. Exposure to Ultrasound for more than 10 minutes damages the vesicles. 10 minutes of sonication resulted in uniform unilamellar vesicles.

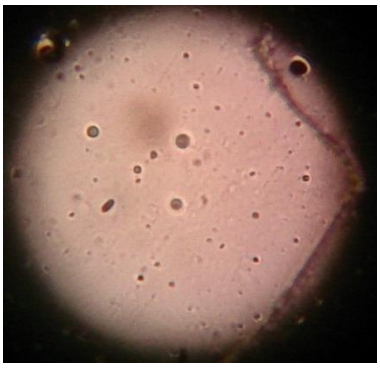
❖ **Effect of Osmotic shock[100]**

The niosomal formulations were treated with hypotonic (0.5% NaCl), hypertonic (1.6% NaCl) and isotonic saline (0.9% NaCl). The effect of osmotic shock is shown in figure.

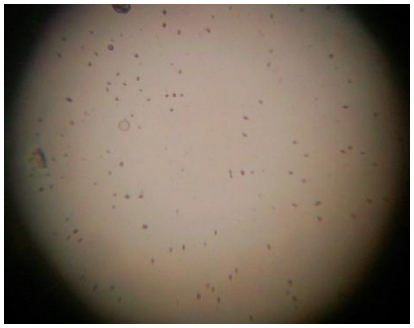
Fig. 22: Effect of Osmotic Shock

a.Hypertonic (1.6% NaCl) **b.Hypotonic (0.5% NaCl)**





c. Isotonic saline (0.9% NaCl)



CHARACTERIZATION OF NIOSOMES

❖ **Drug content and Entrapment Efficiency**

Table 11: Drug Content and Entrapment Efficiency of Ketoconazole Niosomes

FORMULATION CODE	% DRUG CONTENT (% w/w)	% ENTRAPMENT EFFICIENCY (% w/w)
KTZ 20 – 1	94.64	41.7
KTZ 20 – 2	93.41	52.1
KTZ 20 – 3	95.22	56.1
KTZ 20 – 4	91.73	46.2
KTZ 20 – 5	97.70	43.2
KTZ 40 – 1	94.06	51.7
KTZ 40 – 2	87.61	59.1
KTZ 40 – 3	95.37	65.3
KTZ 40 – 4	88.81	72.3
KTZ 40 – 5	92.31	69.2
KTZ 60 – 1	98.44	51.8
KTZ 60 – 2	97.57	52.8
KTZ 60 – 3	95.81	56.21
KTZ 60 – 4	94.64	56.6
KTZ 60 – 5	99.6	60.2

Fig. 23: Drug Content of Ketoconazole Niosomes

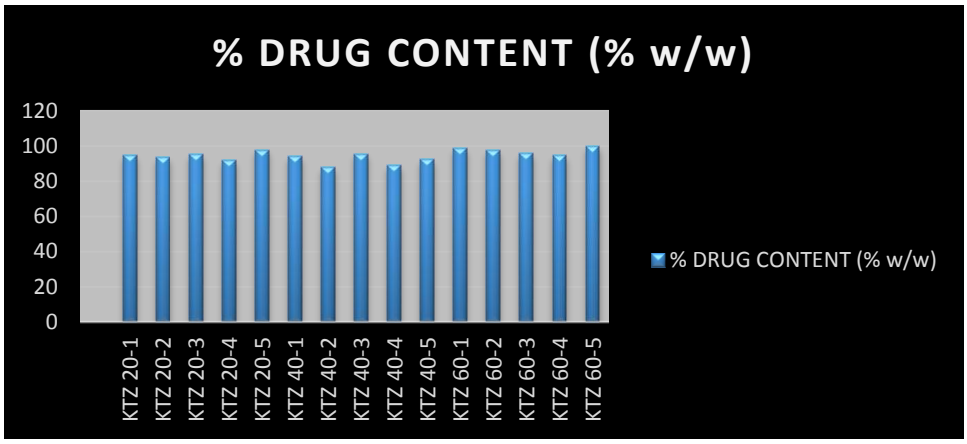
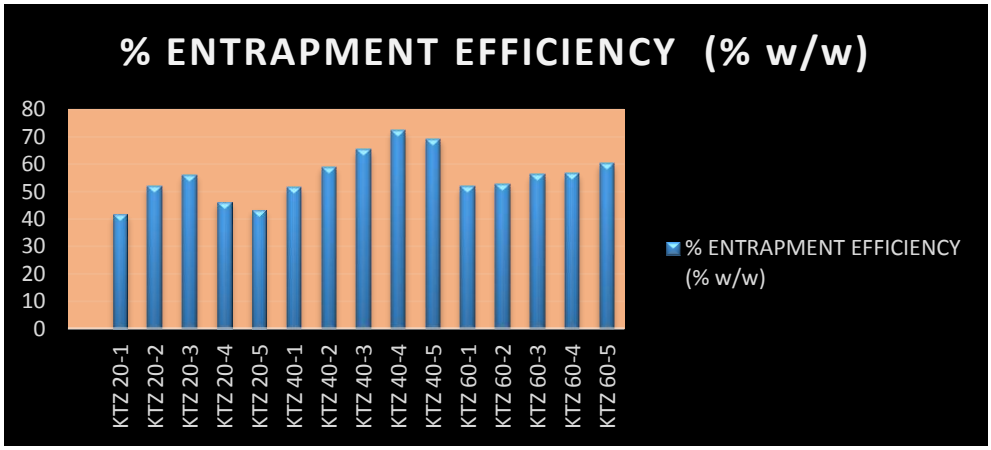


Fig. 24: Entrapment Efficiency of Ketoconazole Niosomes



The entrapment efficiency of the formulations was observed to be between 41.7% and 72.1%. The entrapment efficiency was found to be 56.1%, 72.3% and 60.2% for the formulations KTZ 20-3, KTZ 40-4 and KTZ 60-5.

The highest entrapment efficiency was observed with Span 40 formulation followed by Span 60 niosomes in the similar range. This may be due to the higher HLB value of Span 40 compared to Span 60. The entrapment efficiency decreased as the HLB value decreased from 6.7 of Span 40 to 4.7 of Span 60.[15]Eventhough, Span 20 has a HLB value of 8.6 the entrapment efficiency of the SPAN 20 formulations was observed to be less than the other two formulations. This may be due to the difference in phase transition temperature.[36,132.] The order of non -ionic surfactants that resulted in better entrapment efficiency is as follows:

Span 40 > Span 60 > Span 20

IN- VITRO RELEASE STUDY[33]

The *in- vitro* release study of Ketoconazole niosomes was done using dialysis membrane in an open ended tube- beaker assembly using phosphate buffered saline (PBS) pH 7.4 with 10% methanol as the diffusion medium. The results are shown in Tables and figures

Table 12: *In- vitro* release of niosomes containing Span 20 in different ratios

Time (in hours)	Cumulative % Drug Release (% w/w)					
	Pure Drug	**KTZ 20-1**	KTZ 20-2	**KTZ 20-3**	**KTZ 20-4**	**KTZ 20-5**
0	0	0	0	**0**	0	0
1	10.14	7.21	7.42	**9.31**	7.10	9.48
2	33.72	10.49	11.50	**12.85**	10.30	13.20
3	48.98	17.66	14.54	**15.16**	15.77	16.76
4	62.32	23.61	18.31	**18.12**	20.07	19.69
5	72.21	27.92	21.00	**22.32**	25.25	22.01
6	88.45	30.71	23.17	**27.48**	28.52	25.82
7	94.14	33.57	26.02	**34.65**	31.90	27.64
8	98.32	36.51	28.34	**37.27**	35.38	30.93
9		41.05	32.58	**41.75**	38.25	36.46
10		44.98	36.35	**45.72**	41.20	39.35
11		49.02	40.87	**48.39**	44.22	42.31
12		51.65	43.06	**52.56**	48.73	45.34
24		65.77	69.43	**82.21**	73.99	64.14

The cumulative % drug release at 24 hours increased initially with 65.77% for KTZ 20-1, 69.43% for KTZ 20-2 and 82.21% for KTZ 20-3; after which the release decreased gradually for the next higher surfactant ratios with 73.99% for KTZ 20- 4 and 64.14% for KTZ 20- 5.

Table 13: *In- vitro* release of niosomes containing Span 40 in different ratios

Time (in hours)	Cumulative % Drug Release					
	Pure Drug	**KTZ 40- 1**	**KTZ 40- 2**	**KTZ 40- 3**	**KTZ 40- 4**	**KTZ 40- 5**
0	0	0	0	0	**0**	0
1	10.14	5.85	8.86	7.83	**7.59**	6.74
2	33.72	9.56	11.33	12.47	**11.62**	11.64
3	48.98	11.26	13.63	16.46	**15.47**	14.56
4	62.32	12.41	16.01	18.20	**20.42**	17.60
5	72.21	17.30	21.13	22.41	**26.56**	20.29
6	88.45	23.03	22.29	27.75	**30.94**	23.54
7	94.14	32.72	26.16	31.38	**33.29**	26.44
8	98.32	37.89	30.17	34.16	**35.16**	28.49
9		40.77	32.59	38.46	**38.47**	34.36
10		44.95	34.48	43.39	**43.28**	40.46
11		49.90	36.40	46.56	**45.43**	43.50
12		52.38	40.09	49.80	**48.55**	47.08
24		72.97	74.72	72.58	**82.69**	70.32

The cumulative % drug release at 24 hours for the Span 40 series is 72.97% for KTZ 40 - 1 whereas it is 74.72%, 72.58%, 82.69% and 7.96% for formulations KTZ 40 - 2, KTZ 40 – 3, KTZ 40 – 4, KTZ 40 - 5 respectively.

Table 14: *In- vitro* release of niosomes containing Span 60 in different ratios

Time (in hours)	Cumulative % Drug Release (% w/w)					
	Pure Drug	**KTZ 60- 1**	**KTZ 60-2**	**KTZ 60- 3**	**KTZ 60-4**	**KTZ 60- 5**
0	0	0	0	**0**	0	0
1	10.14	6.74	5.52	**4.72**	4.74	6.67
2	33.72	10.99	10.91	**8.61**	8.65	10.87
3	48.98	13.24	12.48	**12.94**	12.45	14.41
4	62.32	18.18	14.82	**16.35**	15.28	18.60
5	72.21	22.32	16.07	**20.46**	20.48	21.46
6	88.45	23.92	24.37	**25.30**	23.10	23.42
7	94.14	31.46	27.21	**28.65**	32.54	26.43
8	98.32	34.59	30.72	**32.68**	34.54	30.55
9		37.81	33.76	**39.08**	38.25	32.81
10		40.53	37.47	**46.29**	40.94	34.62
11		43.90	40.71	**48.75**	43.14	37.45
12		47.95	44.05	**51.24**	46.48	41.37
24		57.45	63.25	**76.68**	63.96	58.47

The cumulative % drug release of Span 60 niosomes at 24 hours was observed to be increasing in the order as follows; 57.45% for KTZ 60-1, 63.25% for KTZ 60-2 and 76.68% for KTZ 60-3 respectively, after which the cumulative % drug release decreased gradually with 63.96% for KTZ 60-4 and 58.47% for KTZ 60-5 respectively.

Fig. 25: *In- vitro* release of niosomes containing Span 20 in different ratios

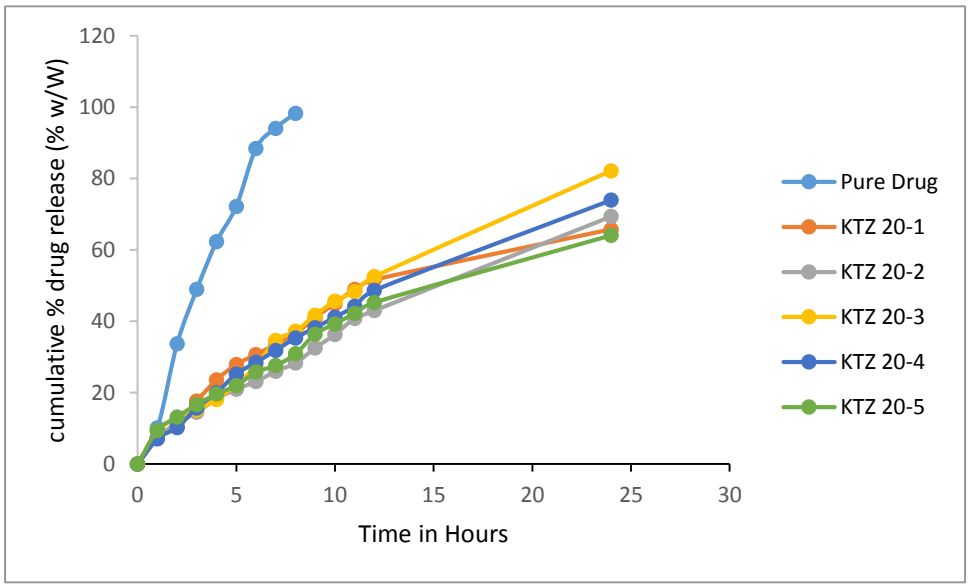


Fig. 26: *In- vitro* release of niosomes containing Span 40 in different ratios

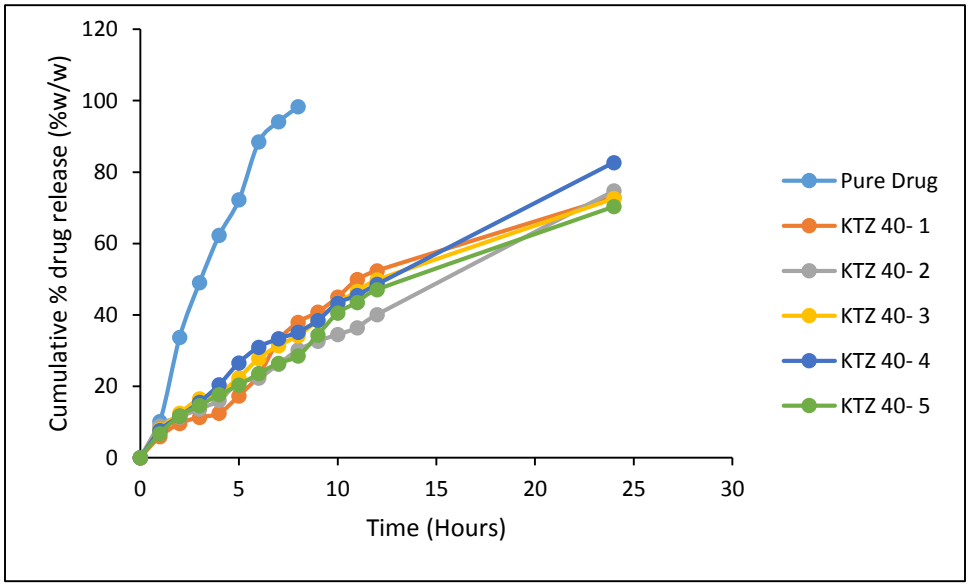


Fig. 27: *In- vitro* release of niosomes containing Span 60 in different ratios

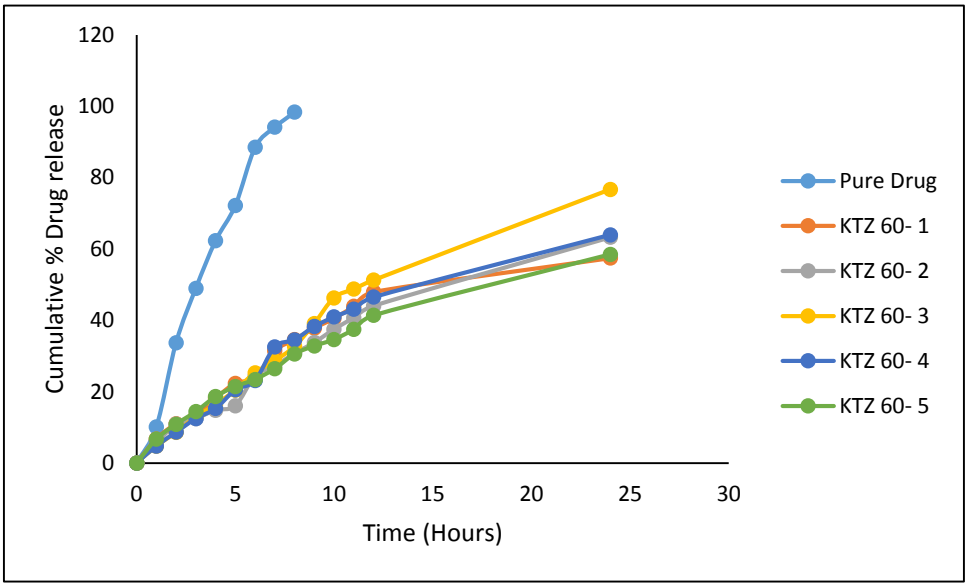
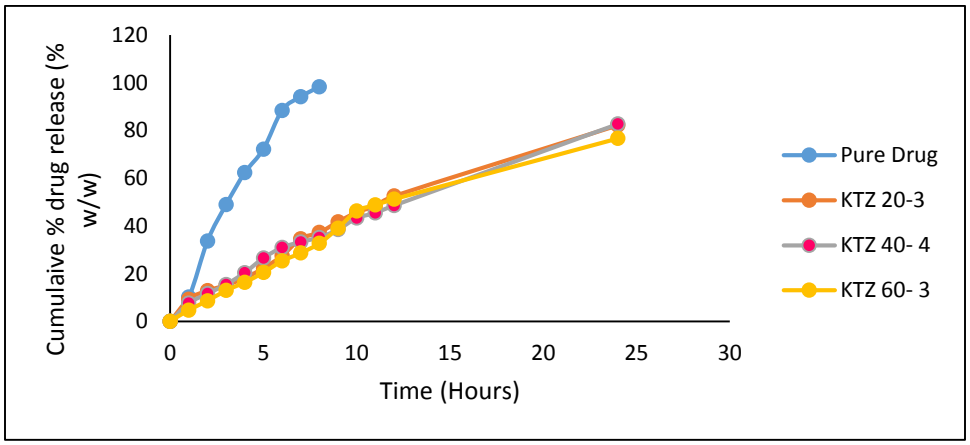


Fig. 28: Comparison of *in- vitro* release of niosomes with highest entrapment efficiency



All the niosomal formulations showed sustained release when compared with pure drug.

The cumulative % drug release at 24 hours for different ratios of the three non-ionic surfactants was in the following order:

Span 20: **KTZ 20- 1 < KTZ 20- 2 < KTZ 20- 3 > KTZ 20- 4 > KTZ 20- 5**
Span 40: **KTZ 40- 1 < KTZ 40- 2 > KTZ 40- 3 < KTZ 40- 4 > KTZ 40- 5**
Span 60: **KTZ 60- 1** < **KTZ 60 -2 < KTZ 60 – 3 > KTZ 60 -4 < KTZ 60 - 5**

KINETICS OF DRUG RELEASE

The *in- vitro* release data was applied to various kinetic models to predict the mechanism of drug release of the niosomal formulations prepared with different non ionic surfactants.

Release Kinetics of Formulation KTZ 20-3

Fig. 29: Zero- Order Release Kinetics of KTZ 20-3

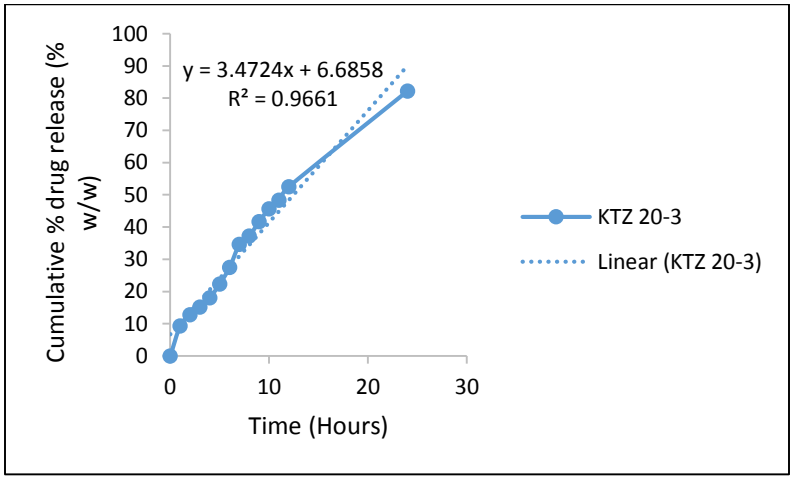


Fig. 30: First Order Release Kinetics of KTZ 20-3

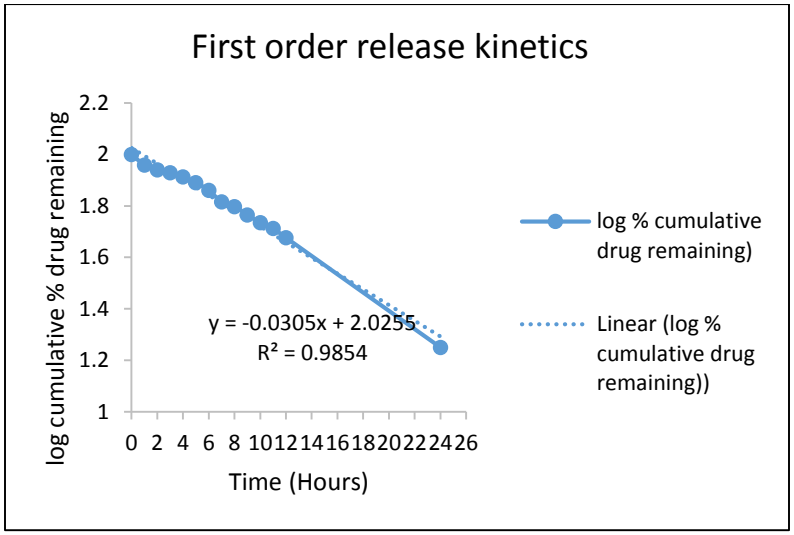


Fig. 31: Higuchi Model Kinetics of KTZ 20-3

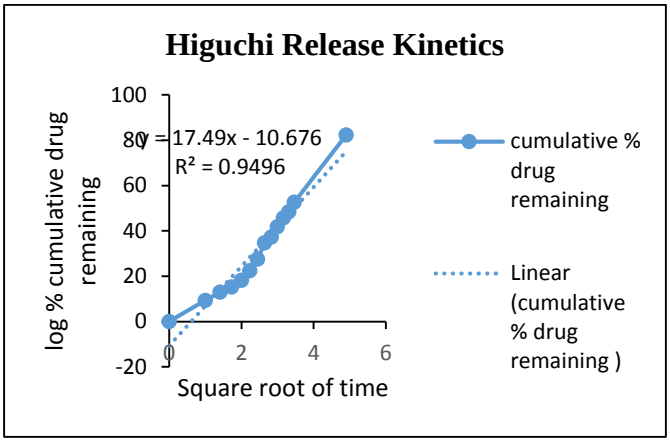


Fig. 32: Hixon Crowell Model Kinetics of KTZ 20-3

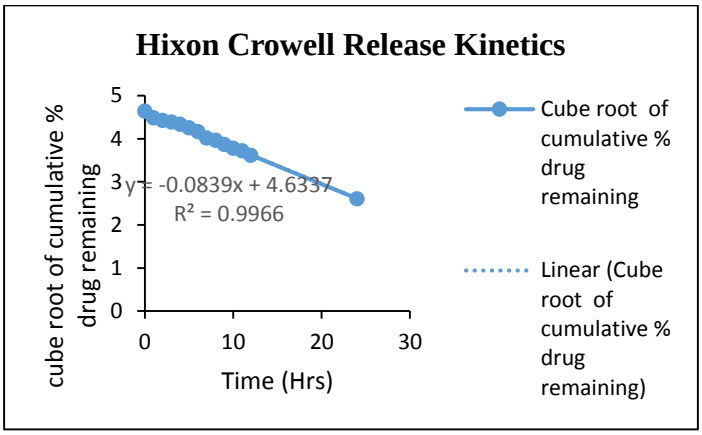
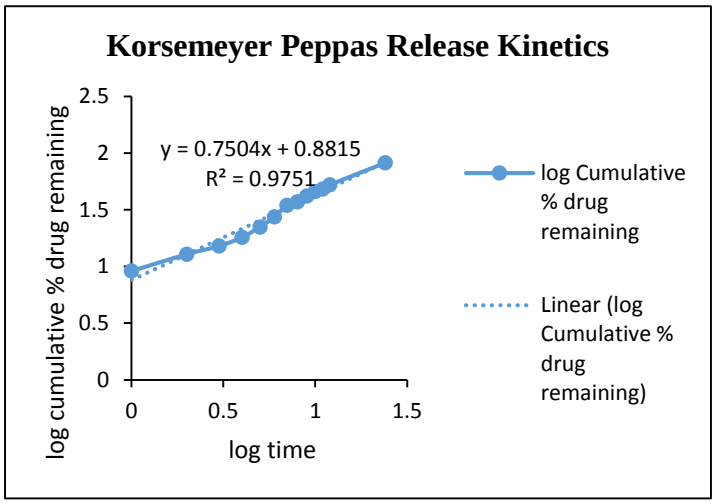


Fig. 33: Korsemeyer- Peppas Model Kinetics of KTZ 20-3



Release Kinetics of Formulation KTZ 40-4

Fig. 34: Zero Order Release Kinetics of KTZ 40-4

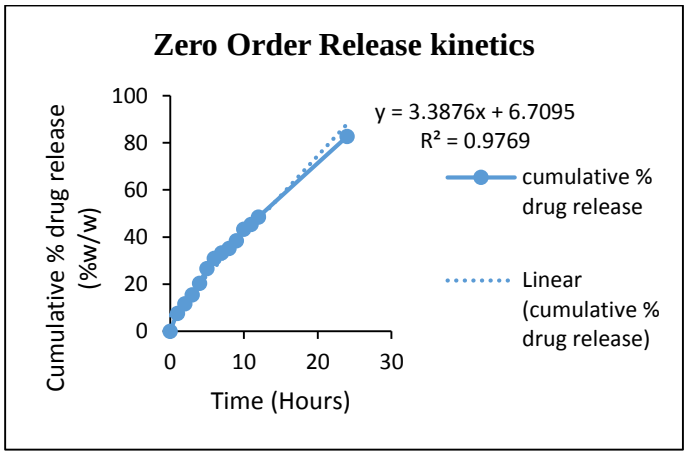


Fig. 35: First Order Release Kinetics of KTZ 40-4

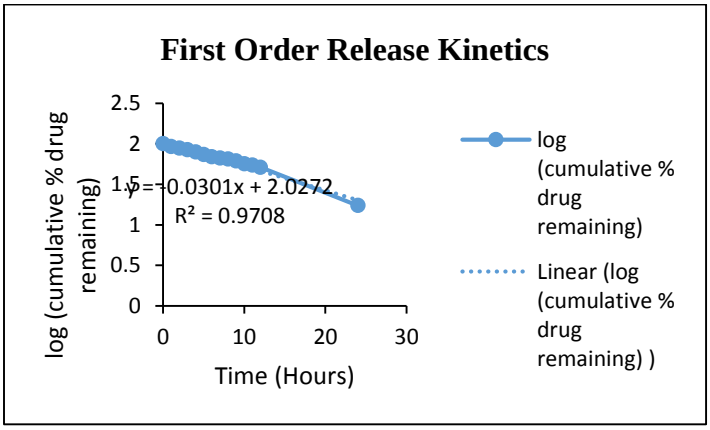


Fig. 36: Higuchi Model Kinetics of KTZ 40-4

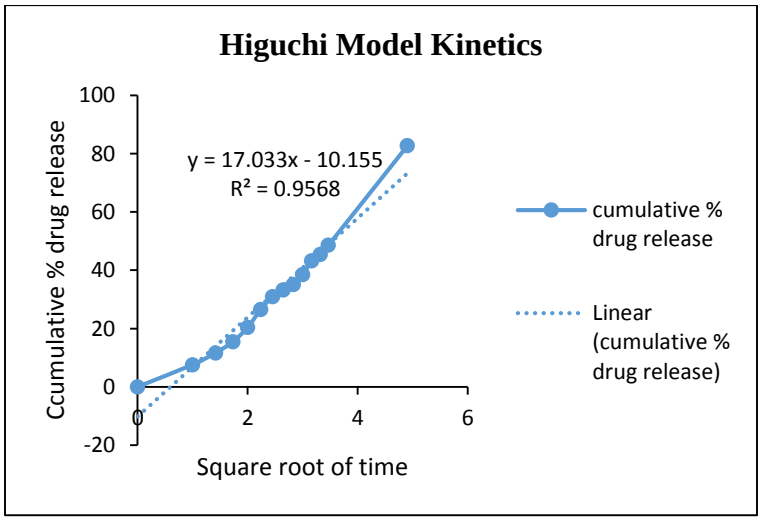


Fig. 37: Hixon Crowell Kinetics of KTZ 40-4

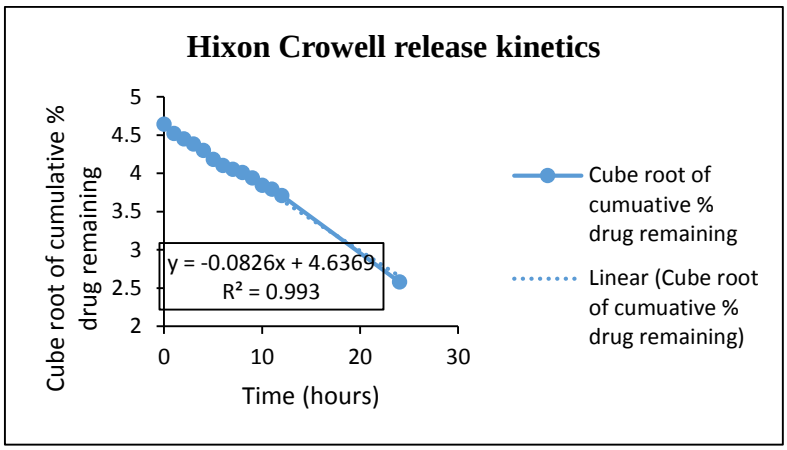
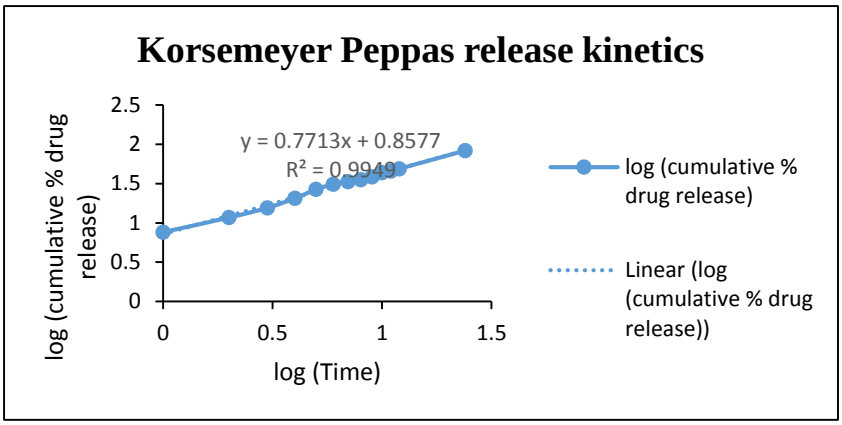


Fig. 38: Korsemeyer- Peppas Model Kinetics of KTZ 40-4



Release Kinetics of Formulation KTZ 60-5

Fig. 39: Zero- Order Release Kinetics of KTZ 60-5

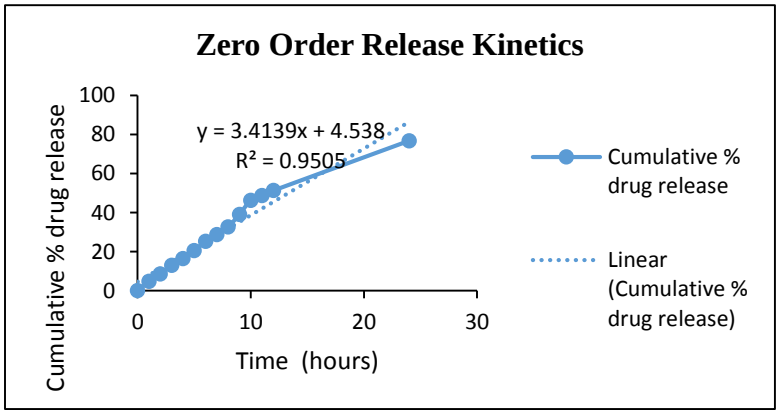


Fig. 40: First Order Release Kinetics of KTZ 60-5

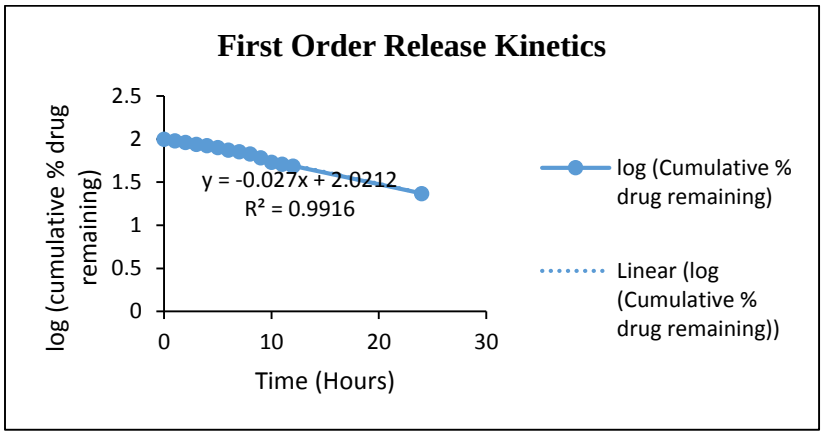


Fig. 41: Higuchi Model Kinetics of KTZ 60-5

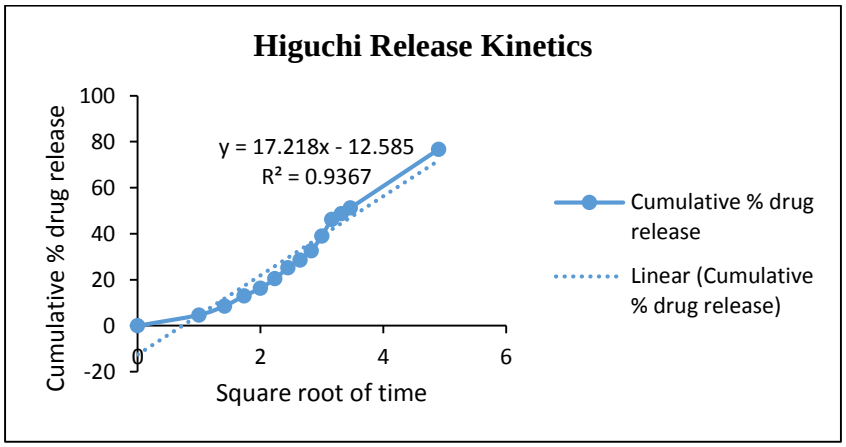


Fig. 42: Hixon Crowell Model Kinetics of KTZ 60-5

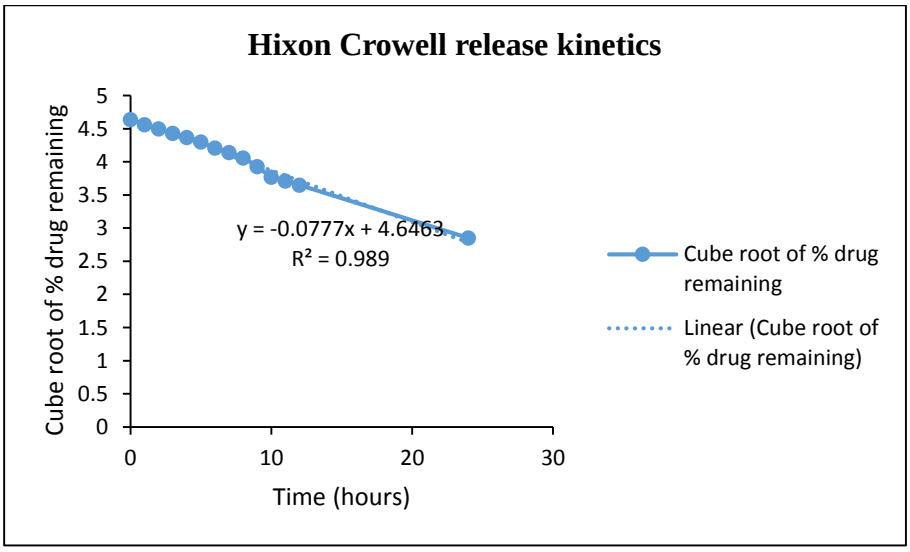
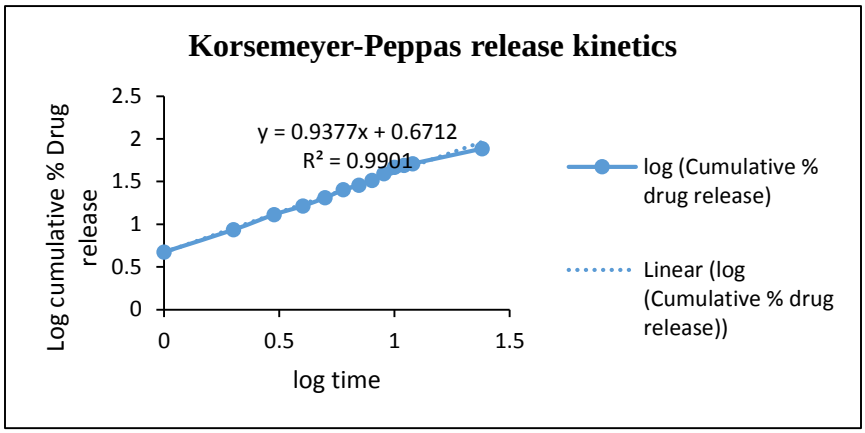


Fig. 43: Korsemeyer- Peppas Release Kinetics of KTZ 60-5



The regression coefficient (r^2) was determined using the drug release data. The results are shown in Table.

Table 15: Drug Release Kinetics

FORMULATION CODE	ZERO ORDER	FIRST ORDER	HIGUCHI MODEL	KORSEMEYER PEPPAS		HIXSON CROWELL
	R^2	R^2	R^2	R^2	N	R^2
KTZ 20-3	0.966	0.985	0.949	0.975	0.750	0.996
KTZ 40-4	0.976	0.970	0.956	0.994	0.771	0.993
KTZ 60-5	0.950	0.991	0.936	0.990	0.937	0.989

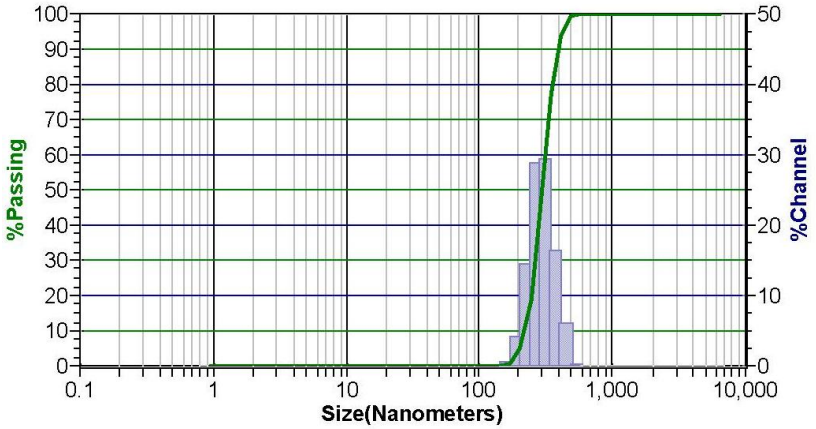
All the formulations followed first- order kinetics and their R^2 value lied between 0.970 and 0.991 indicating the release to be dose dependent.

- The drug release was proportional to the square root of time indicating that Ketoconazole release from niosomes was diffusion controlled.
- The n value for the Korsemeyer- Peppas model for Ketoconazole niosomal formulation was found to be between 0.750 and 0.937 which confirms the anomalous Non- Fickian type diffusion and Super case-II report.

- The drug release pattern from Ketoconazole loaded niosomes follows **Korsemeyer-Peppas model, Higuchi model and first order release.**

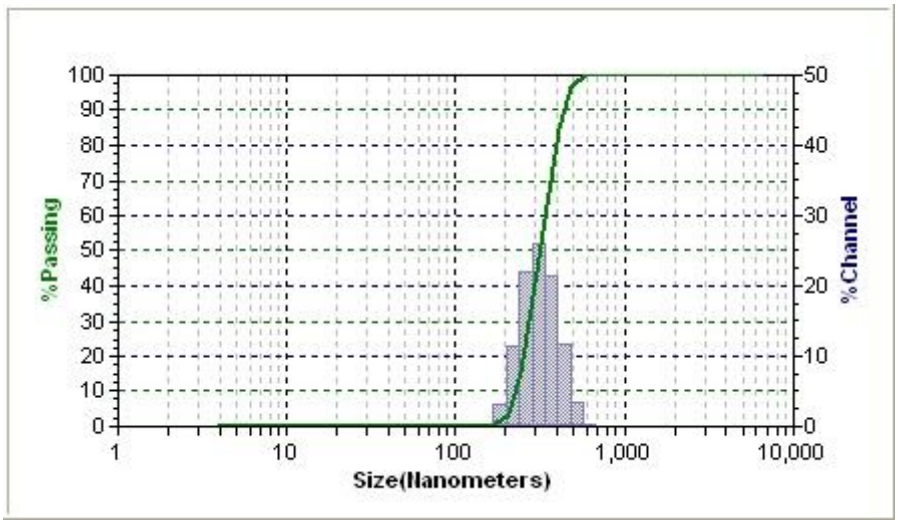
PARTICLE SIZE ANALYSIS

Fig. 44: Particle Size of Formulation KTZ 20-3



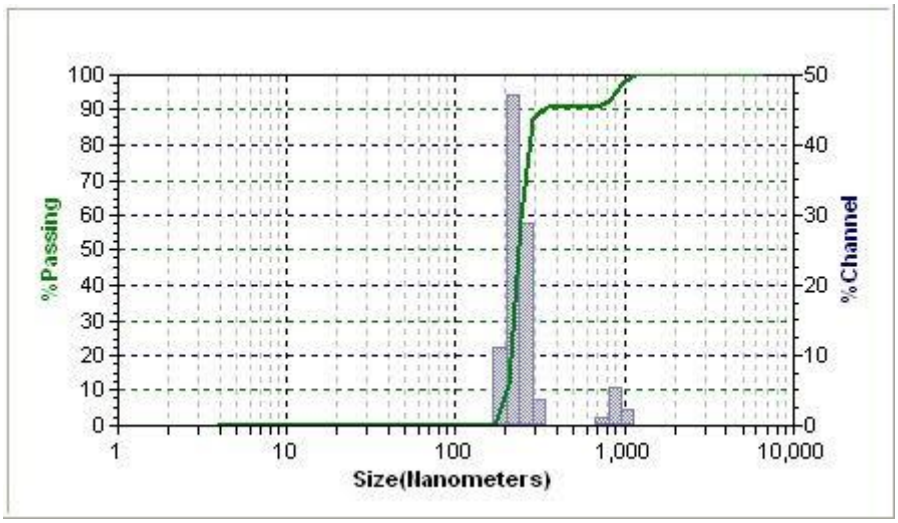
The average particle size of KTZ 20-3 formulation was found to be 260.1nm..

Fig. 45: Particle Size of Formulation KTZ 40-4



The average particle size of KTZ 40-4 formulation was found to be 272.3 nm

Fig. 46: Particle Size of Formulation KTZ 60-5



The average particle size of KTZ 60-5 formulation was found to be 226.3 nm.

/ The average vesicle sizes of the Ketoconazole Niosomes are in the order

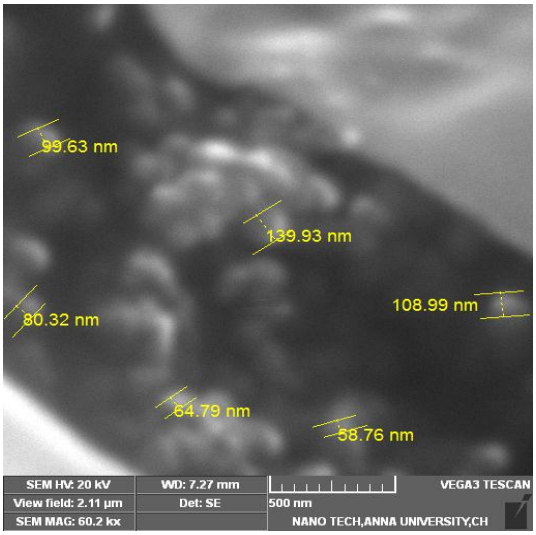
Span 40 > Span 20 > Span 60

SCANNING ELECTRON MICROSCOPY

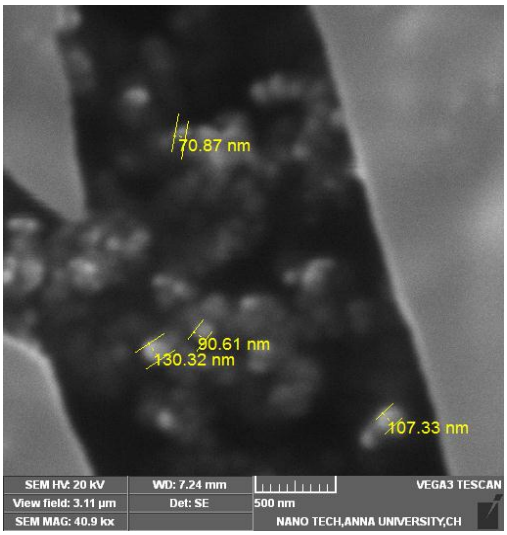
The SEM images were seen for the optimized niosomal formulations. The results are shown in the following figures.

Fig. 47: SCANNING ELECTRON MICROSCOPIC IMAGES OF KETOCONAZOLE NIOSOMES

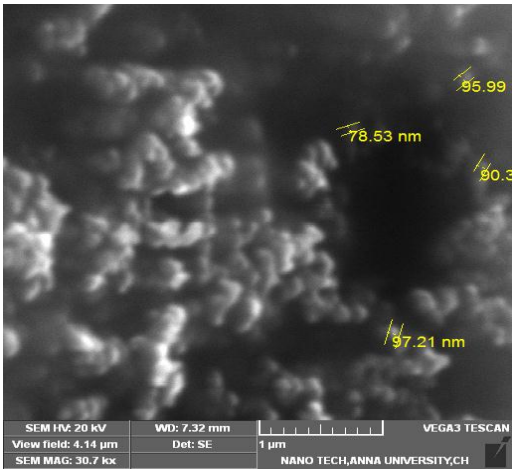
a) Span 20 (KTZ 20-3)



b) Span 60 (KTZ 40-4)



c) Span 60 niosomes (KTZ 60-5)



The SEM analysis showed that the optimized niosomal formulations were discrete and spherical in shape.

FORMULATION OF KETOCONAZOLE NIOSOMAL GEL

Ketoconazole niosomal gel formulations with the optimized ratio of the surfactants were prepared with Carbopol 940 as gelling agent which is due to its hydrophilic nature and bio-adhesive properties, which may result in an increased residence time of drug at the site of absorption by interacting with the mucosa. The formed gel formulations were evaluated for appearance, pH, viscosity, drug content, and *in-vitro* drug diffusion study.

- **Physical appearance:**

  The formed gel is off-white in color. It is little bit sticky in nature.

- **pH:**

  The pH of the gel formulations were found to be in the range of 6.7 to 6.9. It is mentioned in the table 15.

  Table 15: pH of the different Niosomal gel formulations.

S.no	Formulation code	pH
01.	KTZ 20-3	6.59
02.	KTZ 40-4	6.72
03.	KTZ 60-5	6.74

- **Viscosity:**

  The viscosity of the gel formulations were mentioned in the table 16.

  Table 16: Viscosity of the gel formulations.

S.no	Formulation code	Viscosity (cps)
01.	KTZ 20-3	8320
02.	KTZ 40-4	8256
03.	KTZ 60-5	8675

- **Drug content analysis:**

  The percentage drug content of the formulations were mentioned in the table as follows;

  Table 17: % Drug content of the different gel formulations.

S.no	Formulation code	% Drug content (%w/w)
01.	KTZ 20-3	98.13
02.	KTZ 40-4	96.10
03.	KTZ 60-5	94.3

- **Entrapment Efficiency:**

  The entrapment efficiency of the different gel formulations are as follows;

  Table 18: Entrapment Efficiency of the different gel formulations

S.no	Formulation code	% entrapment (%w/w)
01.	KTZ 20-3	52.4
02.	KTZ 40-4	63.8
03.	KTZ 60-5	65.2

The highest entrapment efficiency was observed with KTZ 60-5 formulation. This may be due to the higher phase transition temperature of Span 60.[100,132]

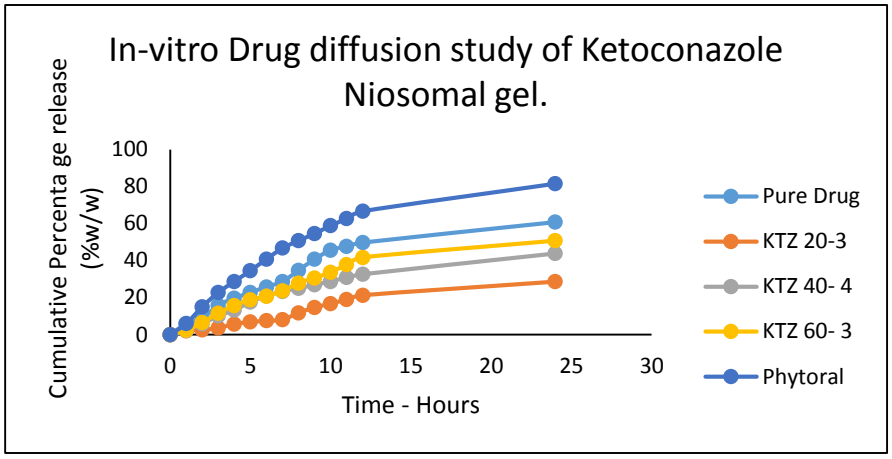
- ***In-vitro* Drug Diffusion studies:**

  The *in-vitro* drug diffusion study of Ketoconazole niosomal gel was done using dialysis membrane in an open ended tube-beaker assembly using phosphate buffered saline (PBS) pH 7.4 as the diffusion medium. The results are shown in tables and figures.

Table 19: *In-vitro* release of Ketoconazole niosomal gel in the optimized ratio of surfactants.

Time (Hours)	Cumulative % Drug release				
	Plain Ketoconazole Gel	KTZ 20-3	KTZ 40-4	KTZ 60-5	Phytoral
0	0	0	0	0	0
1	5.96	2.08	2.28	2.47	6
2	8.9	2.7	5.91	6.6	15.1
3	15.97	3.63	10.2	11.65	22.84
4	19.73	5.8	13.29	15.81	28.73
5	22.84	6.87	17.71	18.95	34.66
6	25.86	7.58	20.92	20.81	40.82
7	28.78	8.11	23.45	23.72	46.81
8	34.79	11.85	25.06	27.93	50.81
9	40.84	14.76	26.96	30.7	54.71
10	45.72	16.79	28.78	33.75	58.9
11	47.76	19.09	30.9	37.89	62.79
12	49.81	21.37	32.67	41.77	66.76
24	60.87	28.72	43.87	50.77	81.61

Figure.48. *In-vitro* Drug diffusion study of Ketoconazole Niosomal gel.



The cumulative percentage release for the plain ketoconazole gel, marketed ointment, niosomal gel preparations at the end of 24 hours were **plain gel** -60.87, **phytoral**-81.61, **KTZ 20-3** -28.72, **KTZ 40-4**-43.87, **KTZ 60-5** – 50.77 respectively. The niosomal gel preparations showed prolonged release at the end of 24 hours.

KINETICS OF DRUG RELEASE

The *in- vitro* release data was applied to various kinetic models to predict the mechanism of drug release of the niosomal gel formulations prepared with different non ionic surfactants.

Release Kinetics of Formulation KTZ 20-3 gel

Fig. 49: Zero- Order Release Kinetics of KTZ 20-3.

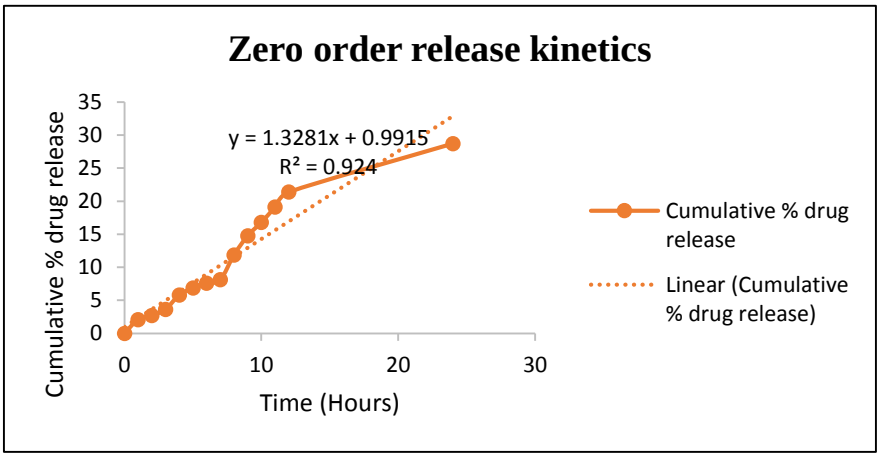


Fig. 50: First Order Release Kinetics of KTZ 20-3

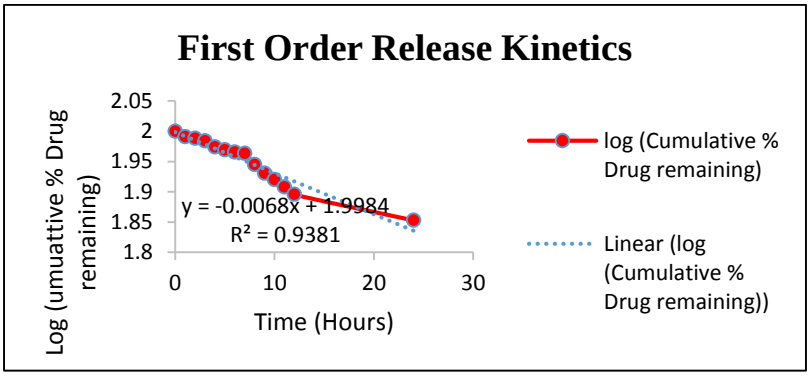


Fig. 51: Higuchi Model Kinetics of KTZ 20-3

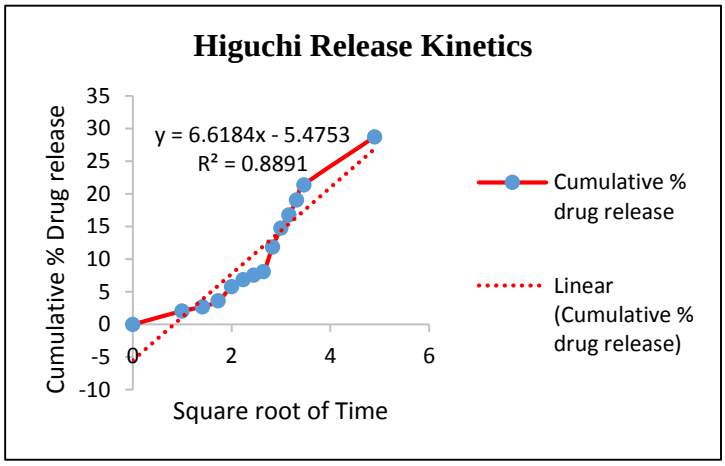


Fig. 52: Hixon Crowell Model Kinetics of KTZ 20-3

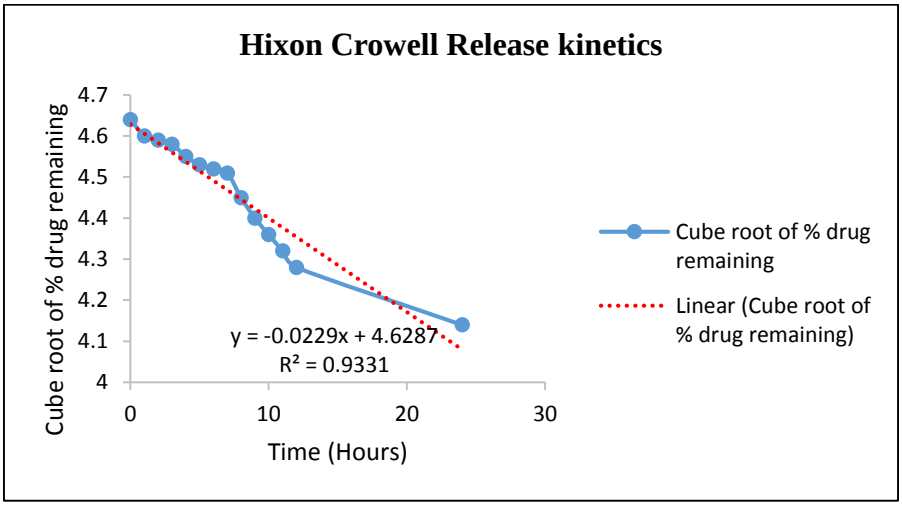
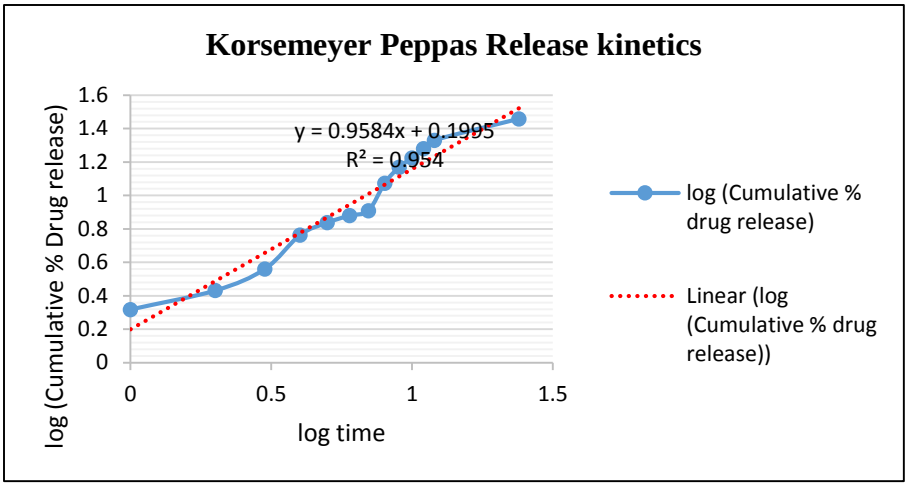


Fig. 53: Korsemeyer- Peppas Model Kinetics of KTZ 20-3



Release Kinetics of Formulation KTZ 40-4 gel

Fig. 54: Zero Order Release Kinetics of KTZ 40-4

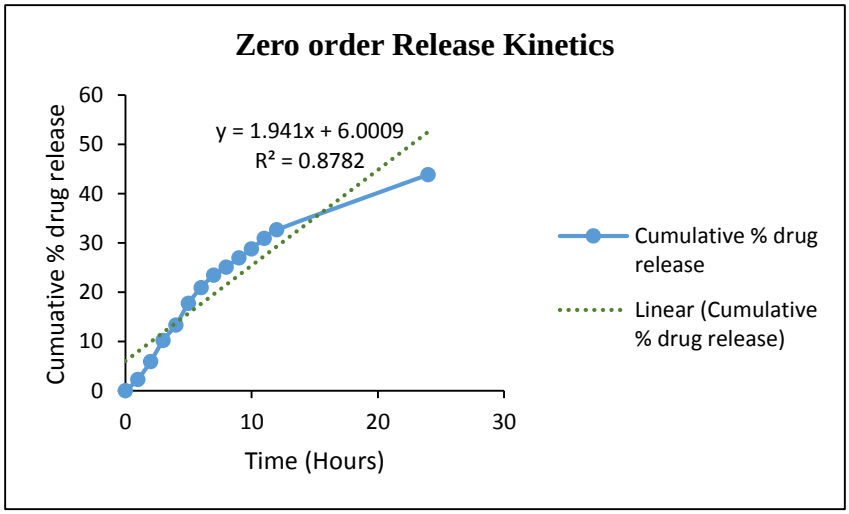


Fig. 55: First Order Release Kinetics of KTZ 40-4

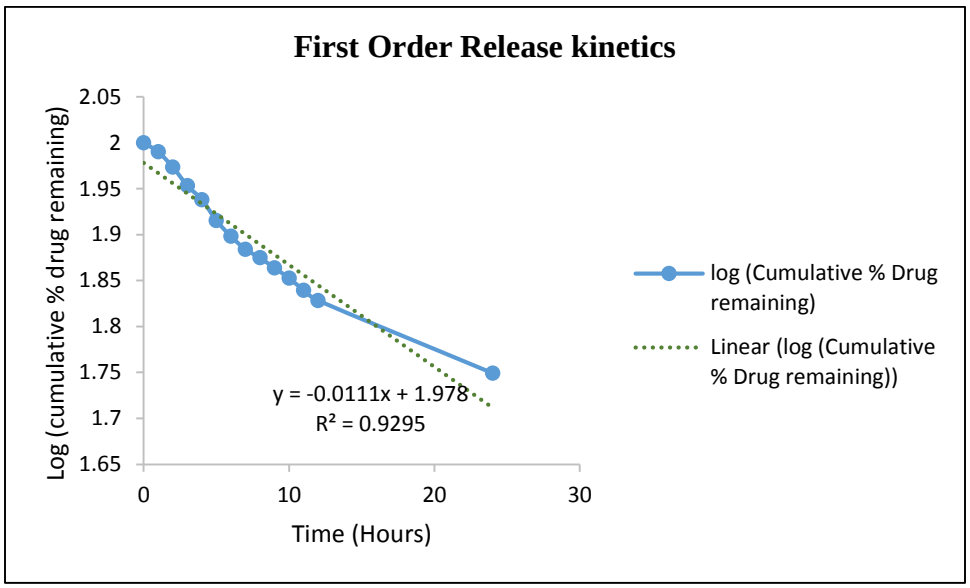


Fig. 56: Higuchi Model Kinetics of KTZ 40-4

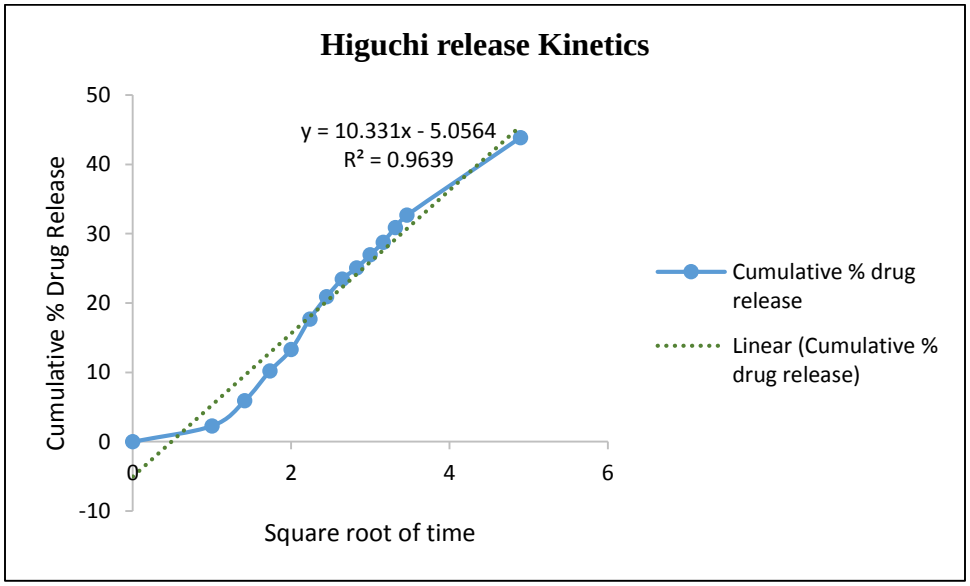


Fig. 57: Hixon Crowell Kinetics of KTZ 40-4

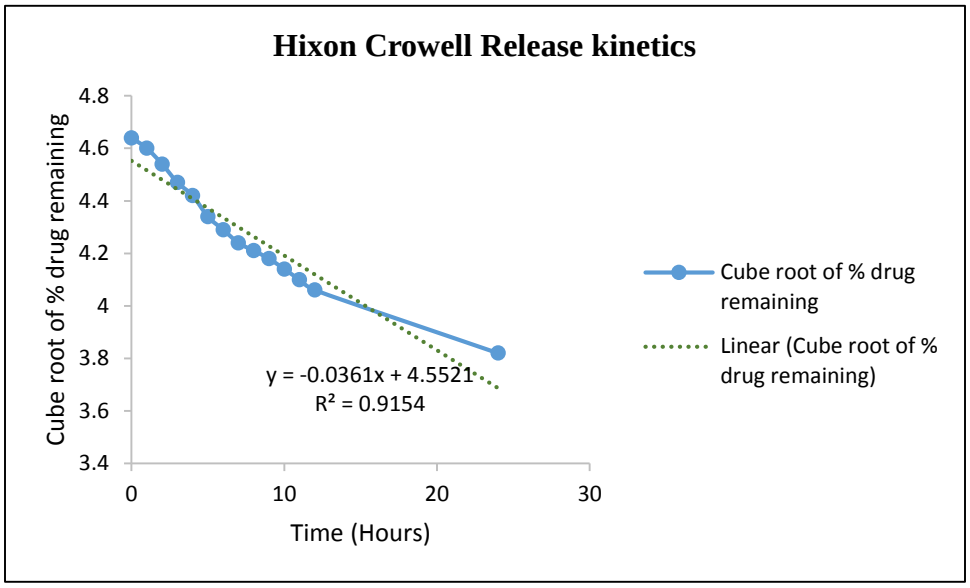
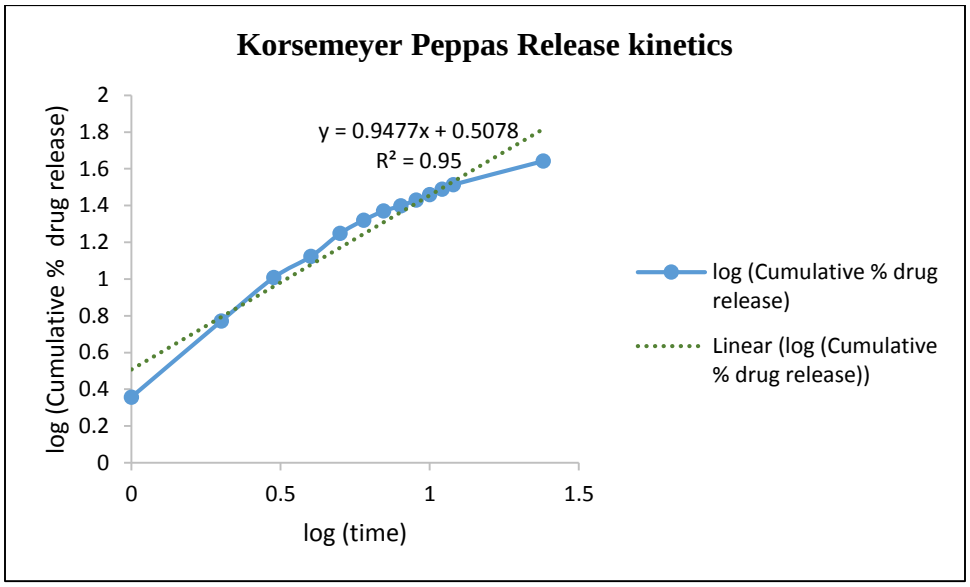


Fig. 58: Korsemeyer- Peppas Model Kinetics of KTZ 40-4



Release Kinetics of Formulation KTZ 60-5 gel

Fig. 59: Zero- Order Release Kinetics of KTZ 60-5

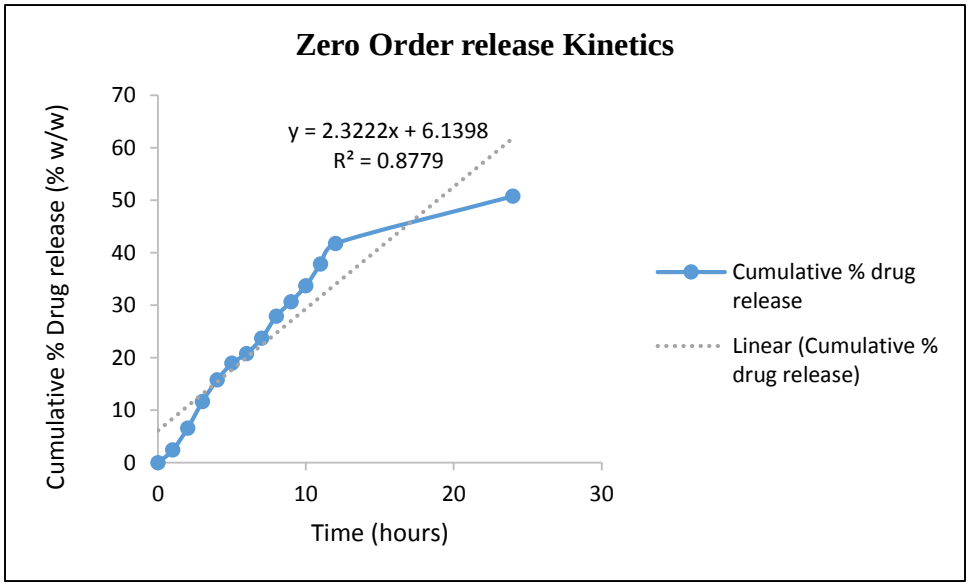


Fig. 60: First Order Release Kinetics of KTZ 60-5

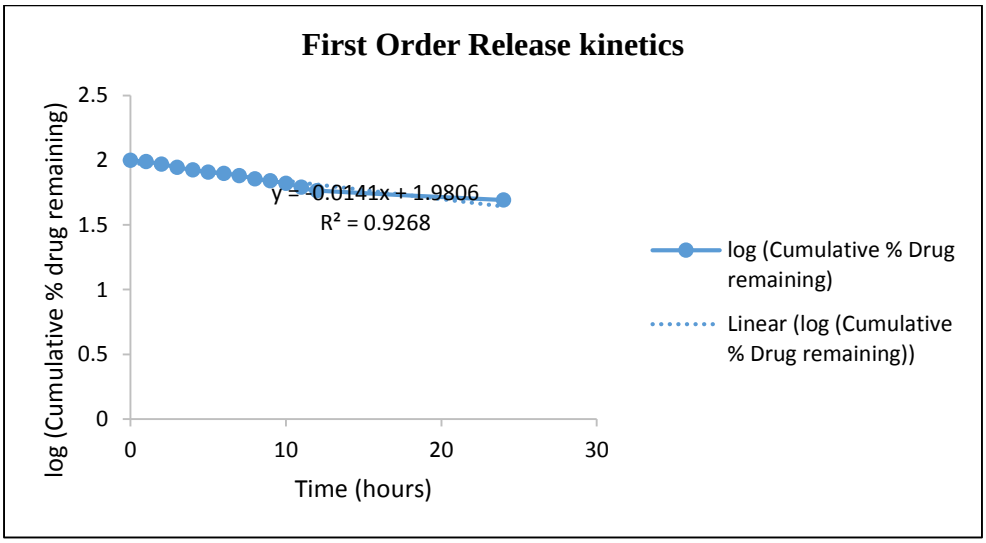


Fig. 61: Higuchi Model Kinetics of KTZ 60-5

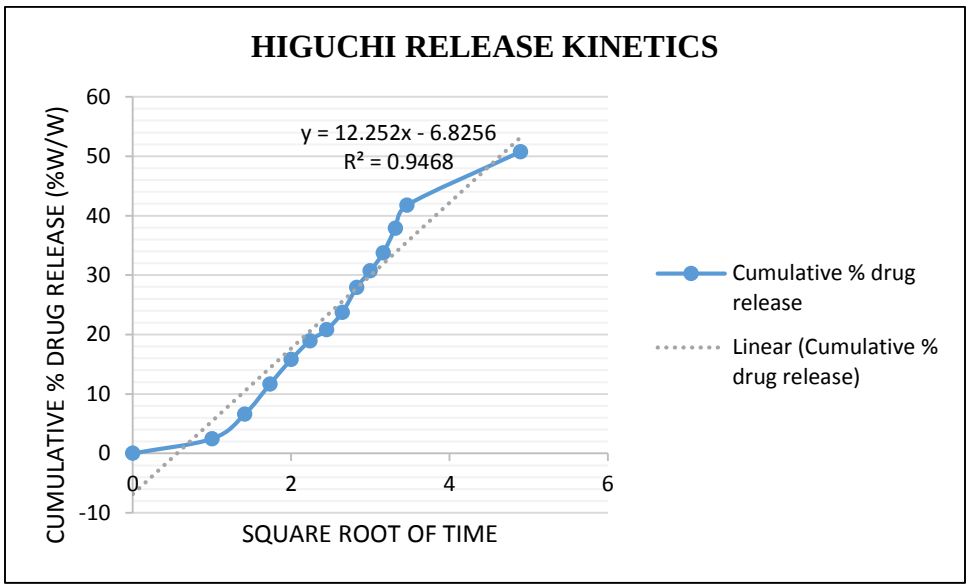


Fig. 62: Hixon Crowell Model Kinetics of KTZ 60-5

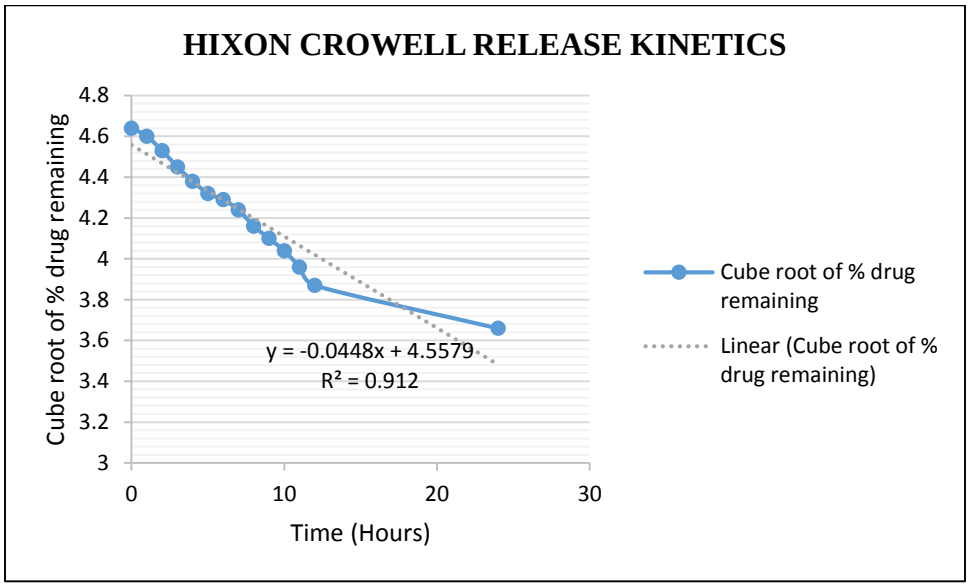
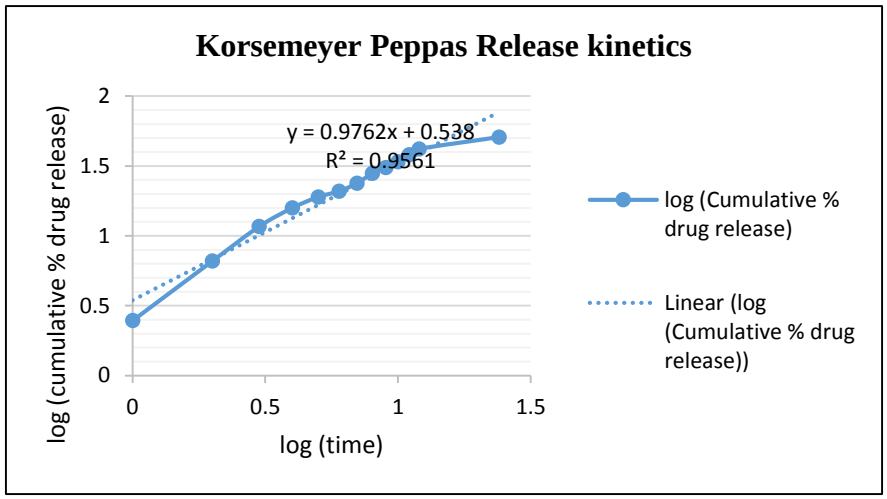


Fig. 63: Korsemeyer- Peppas Release Kinetics of KTZ 60-5



The regression coefficient (r^2) was determined using the drug release data. The results are shown in Table.

Table 20: Drug Release Kinetics

FORMULATION CODE	ZERO ORDER	FIRST ORDER	HIGUCHI MODEL	KORSEMEYER PEPPAS		HIXSON CROWELL
	R^2	R^2	R^2	R^2	N	R^2
KTZ 20-3	0.929	0.938	0.889	0.954	0.958	0.933
KTZ 40-4	0.878	0.929	0.963	0.95	0.947	0.915
KTZ 60-5	0.877	0.926	0.946	0.956	0.976	0.912

All the formulations followed first- order kinetics and their R^2 value lied between 0.926 and 0.938 indicating the release to be dose dependent.

- The drug release was proportional to the square root of time indicating that Ketoconazole release from niosomes was diffusion controlled.
- The n value for the Korsemeyer- Peppas model for Ketoconazole niosomal formulation was found to be between 0.947 and 0.976 which confirms the Super case-II transport.

- The drug release pattern from Ketoconazole loaded niosomal gel follows **Korsemeyer-Peppas model, Higuchi model and first order release.**

STABILITY STUDIES OF KETOCONAZOLE NIOSOMAL GEL [50,100]

Stability studies of the ketoconazole gel formulations (KTZ 20-3, KTZ 40-4, KTZ 60-5) were carried out by storing at 4°C - 8°C (refrigeration temperature) and 25°C ±2°C for a period of 45 days as per ICH (International Conference on Harmonization) guidelines.

Table 21: Stability study of ketoconazole niosomal gel formulation KTZ 20-3 at different temperature.

Time of storage in days	Temperature of storage			
	Drug Content (%) 4°C - 8°C (refrigeration temperature)	Entrapment efficiency (%) 4°C - 8°C (refrigeration temperature)	Drug Content (%) 25°C ±2°C (room temperature)	Entrapment efficiency (%) 25°C ±2°C (room temperature)
0	98.13	52.4	98.13	52.4
15	98.04	50.1	94.94	47.5
30	97.84	49.6	92.8	46.3
45	97.6	49.3	91.8	45.9

Table 22: Stability study of ketoconazole niosomal gel formulation KTZ 40-4 at different temperature.

Time of storage in days	Temperature of storage			
	Drug Content (%) 4°C - 8°C (refrigeration temperature)	Entrapment efficiency (%) 4°C - 8°C (refrigeration temperature)	Drug Content (%) 25°C ±2°C (room temperature)	Entrapment efficiency (%) 25°C ±2°C (room temperature)
0	96.10	63.8	96.10	63.8
15	95.90	63.5	95.10	63.0
30	95.71	63.2	94.16	62.0
45	995.32	63.0	93.58	61.4

Table 23: Stability study of ketoconazole niosomal gel formulation KTZ 60-5 at different temperature.

Time of storage in days	Temperature of storage			
	Drug Content (%) 4°C - 8°C (refrigeration temperature)	Entrapment efficiency (%) 4°C - 8°C (refrigeration temperature)	Drug Content (%) 25°C ±2°C (room temperature)	Entrapment efficiency (%) 25°C ±2°C (room temperature)
0	94.30	65.2	94.30	65.2
15	94.10	64.7	93.19	64.3
30	93.97	64.2	92.22	63.1
45	93.3	63.1	91.06	62.0

- The entrapment efficiency of the drug in the niosomal gel was estimated immediately after the preparation and after every 15 days for 45 days . The drug leakage from the vesicles was least at 4°C. this may be attributed to phase transition of surfactant and lipid causing leakage of vesicles occurs at higher temperature at storage. Hence, the niosomes can be stored at 4-8°C. The improved stability of niosomes after incorporation into the gel base may be due to prevention of fusion of niosomes. The higher drug skin retention in case of niosomal gel maybe due to creation of reservoir effect of drug in the skin and thereby increasing the drug retention capacity into the skin.[50]

10. SUMMARY AND CONCLUSION

- The purpose of this research was to prepare Ketoconazole loaded niosomes for sustained release of drug and incorporate it in to topical gel delivery system to reduce the side effects by site specific targeting.

- Thin film hydration technique was employed to produce niosomes using non ionic surfactants and cholesterol.

- The process related parameters were optimized such as hydration time (60 minutes), sonication time (10minutes), osmotic shock, rotational speed of the evaporator flask (initially 100 rpm, later 150 rpm).

- Cholesterol used as a membrane additive, acts as a stabilizer as well as fluidity buffer to improve the stability of the vesicles.

- The formulations were prepared using different non- ionic surfactants by varying the surfactant concentration (Span 20, Span 40 and Span 60) and keeping the cholesterol concentration fixed.

- The formulated niosomes were characterized for entrapment efficiency.

- The order of entrapment efficiency of the formulations are as follows;
  SPAN 40 > SPAN 60 > SPAN 20

- The order of *in- vitro* release of the formulations are as follows:
  SPAN 40 > SPAN 20 > SPAN 60

- The drug release pattern from Ketoconazole loaded niosomes follows **Higuchi's model First order of release and Korsemeyer Peppas release.**

- The average particle size of the formulated Ketoconazole niosomes exhibited around 260 nm.

- SEM analysis of the niosomal dispersion showed the spherical shape of the vesicles.

- The results of the FT- IR studies proved that there is no interaction between the drug, cholesterol and the non-ionic surfactants.
- The optimized Niosomal dispersion were further formulated as topical gel delivery sysem.
- The formulated niosomal gel was characterized for the physical appearance, pH and rheological behavior.
- The formulated niosomal gel was evaluated for drug content and *in-vitro* diffusion studies were carried out using open ended diffusion tube.
- The order of *in- vitro* release of the niosomal gel formulations are as follows:
  SPAN 60 > SPAN 40 > SPAN 20.
- The drug release pattern from Ketoconazole loaded niosomal gel follows **Higuchi's model First order of release and Korsemeyer Peppas release.**
- From the stability studies, the optimum storage condition for the niosomal gel formulations was found to be 4-8°C
- It is concluded that the thin film hydration technique is a useful method for the successful incorporation of poorly water soluble drug Ketoconazole into niosomes with high entrapment efficiency. The prolonged release of the drug from the niosome suggests that the frequency of administration and adverse effects significantly thereby improving the patient compliance. The administration of drug as gel type formulation enhances its penetration across the stratum corneum and the reduced size and its vesicular structure not only improved its localization in the stratum spinosum in which the infected macrophages reside and also its uptake by the infected macrophages.

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